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(54) Title: DIFFERENTIAL ASSAY FOR ULCERATIVE COLITIS, PRIMARY SCLEROSING CHOLANGITIS AND TYPE 1 AUTOIMMUNE HEPATITIS

(57) Abstract

The invention is directed to methods and kits for detecting and measuring the presence or absence of perinuclear anti-neutrophil cytoplasmic antibody of ulcerative colitis, primary sclerosing cholangitis or type 1 autoimmune hepatitis. The methods and kits of the present invention provide safe and reliable means for diagnosing ulcerative colitis, primary sclerosing cholangitis, and type 1 autoimmune hepatitis. The antigens reactive with perinuclear anti-neutrophil cytoplasmic autoantibody of ulcerative colitis and primary sclerosing cholangitis are also provided.

DIFFERENTIAL ASSAY FOR ULCERATIVE COLITIS, PRIMARY SCLEROSING CHOLANGITIS AND TYPE I AUTOIMMUNE HEPATITIS

5   This is a continuation-in-part of prior pending United States patent application number 08/320,163 filed on October 7, 1994, which is a continuation of the now abandoned United States patent application number 08/028,784 filed on March 10, 1993.

10   ## FIELD OF THE INVENTION

This invention relates to methods and kits for detecting and measuring the presence or absence of perinuclear anti-neutrophil cytoplasmic antibodies of ulcerative colitis, primary sclerosing cholangitis, or type

15   1 autoimmune hepatitis. More specifically, the methods and kits of the present invention employ DNase treatment of neutrophils in assays such as ELISA and immunofluorescence to elicit the loss of a positive control value when the antibody is present.

20   ## BACKGROUND OF THE INVENTION

Inflammatory Bowel Disease (IBD) is the collective term used to describe two gastrointestinal disorders, ulcerative colitis ("UC") and Crohn's disease ("CD"). IBD occurs world-wide and is reported to afflict as many as two million

25   people. Onset has been documented at all ages; however, IBD predominately affects young adults.

The three most common presenting symptoms of IBD are diarrhea, abdominal pain, and fever. The diarrhea may range from mild to severe and is often accompanied by urgency and

frequency. In UC, the diarrhea is usually bloody and may contain mucus and purulent matter as well. Anemia and weight loss are additional common signs of IBD.

A battery of laboratory, radiological, and endoscopic evaluations are combined to derive a diagnosis and to assess the extent and severity of the disease. Nevertheless, differentiating UC from CD, as well as other types of inflammatory conditions of the intestines, such as irritable bowel syndrome, infectious diarrhea, rectal bleeding, radiation colitis, and the like, is difficult. Indeed, depending on the period of follow-up time, in many patients the colitis must be regarded as indeterminate or cannot be definitively diagnosed because of the overlapping features of UC and CD, particularly with CD of the colon.

The selective identification of UC as opposed to CD or other inflammatory conditions of the intestines carries important prognostic and therapeutic implications. For example, when colectomy is indicated, the type of IBD involved determines which surgical options are appropriate. Surgery (total colectomy) does represent a cure in UC, though a dramatic one. In CD, surgery is never curative. Continent procedures such as the ileorectal pull-through (mucosal proctectomy) or the Kock pouch may be desirable in UC, but are contraindicated in CD.

The availability of a diagnostic marker that would readily distinguish UC from CD of the colon and other colitides would represent a major clinical advance. A convenient and reliable blood test which might parallel disease activity or even predict an impending flare of activity would provide a tremendous advantage in the therapeutic management of IBD and aid in the design of more specific treatment modalities.

Although the cause(s) of UC and CD not known, there is general agreement that the immune system is responsible for

mediating the tissue damage in these diseases. A wide range
of immunologic abnormalities have been reported in these
disorders, but none has yet been sufficiently reliable to be
of diagnostic value.

5   A variety of autoantibodies has been observed in UC
patients. Most notable among these antibodies have been
lymphocytotoxic antibodies and colonic epithelial
antibodies. Although these may have genetic and
pathophysiologic implications, they have not been useful

10   diagnostically either because of low frequency of occurrence
or lack of specificity.

        Two other inflammatory diseases which are also
suspected of having autoimmune eitologies are primary
sclerosing cholangitis ("PSC") and type 1 autoimmune

15   hepatitis ("Type 1 AIH"). Like UC and CD, these liver
diseases share common outward symptoms necessitating the use
of invasive technologies, such as liver biopsy and/or ERCP
to identify distinguishing liver abnormalities associated
with AIH and PSC.

20   PSC is characterized by obliterative inflammatory
fibrosis of the extrahepatic bile ducts with or without
involvement of the intrahepatic ducts. The disease
generally progresses in an unrelenting, albeit
unpredictable, fashion to cirrhosis, portal hypertension,

25   and death from liver failure. PSC can occur alone or in
association with UC and less commonly with a variety of
other diseases. Symptoms commonly include jaundice, puritis
and nonspecific upper abdominal pain. Medical treatment of
PSC has included corticosteroids, antibiotics,

30   immunosuppressants, and cholecystogues alone or in
combination. In general, results with all have been
disappointing.

        AIH is a disorder of unknown etiology in which
progressive destruction of the hepatic parenchyma occurs,

often progressing to cirrhosis, and in the more severe
cases, it carries a high mortality rate if untreated.
Although this disease is predominant in woman, it also
affects men. Easy fatigability is the most common symptom at
presentation, and up to 77% of patients also describe
features of jaundice, mild upper abdominal discomfort,
pruritus, anorexia, polymyalgias, diarrhea, and delayed
menarche or amenorrhea are frequent complaints. Cosmetic
change, including facial rounding, hirsutism, and acne. The
histologic hallmark of AIH is periportal, or piecemeal,
necrosis. The condition is considered incurable with a
poor prognosis; spontaneous or sustained remission if
considered rare. Combined treatment prednisone and
azathioprine has been reported to significantly improve life
expectancy and normalize clinical, biochemical and
immunochemical abnormalities. Type 1 AIH is the most common
form of autoimmune hepatitis in the United States, and it is
associated with smooth-muscle antibody or antinuclear
antibody seropositivity, hypergammaglobulinemia, concurrent
immunologic disorders, HLA positivity for A1, B8, DR3, or
DR4, and responsive to corticosteroid therapy.

p-ANCA recently has been demonstrated to be associated
with both Type 1 AIH and PSC. It is reported that p-ANCA
has been found in up to 70% of PSC patient sera while up to
92% of the sera of patients with well defined Type 1 AIH
were found to express high titer pANCA. However, this
discovery has been of limited clinical applicability in the
diagnosis of these hepatobiliary inflammatory diseases due
to an inability to differentiate bewteen the p-ANCA
associated with each disease.

Accordingly, there has existed a need for a convenient
and reliable method to distinguish UC from CD of the colon,
and PSC from Type 1 AIH for diagnostic, prognostic and
therapeutic purposes.

SUMMARY OF THE INVENTION

The present invention provides methods of detecting and measuring the presence or absence of perinuclear anti-neutrophil cytoplasmic antibodies ("p-ANCA") of ulcerative colitis ("UC"), primary sclerosing cholangitis ("PSC") or type 1 autoimmune hepatitis ("Type 1 AIH") in a sample. More specifically, the presence of p-ANCA of UC, PSC or Type 1 AIH is detected by assaying for the loss of a positive value (i.e., loss of a detectable marker as compared to a control) upon treatment of neutrophils with DNase. The present invention demonstrates that the p-ANCA associated with Type 1 AIH is different from that associated with PSC and that each of those p-ANCA are different than the p-ANCA related to UC. These differences can be relied upon to screen for each of the p-ANCA, the p-ANCA associated disease, and to differentiate between the three.

In one embodiment of the present invention, methods of measuring the presence or absence of perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) associated with ulcerative colitis, primary sclerosing cholangitis, or type 1 autoimmune hepatitis in a sample, comprising contacting the sample and a detectable secondary antibody with fixed, DNase-treated neutrophils under conditions suitable to form a complex of neutrophil, p-ANCA and detectable secondary antibody, separating unbound secondary antibody from the complex, and assaying for the pattern of p-ANCA immunoreactivity by detecting the presence, absence or pattern of complexed secondary antibody, compared to a control. DNase treatment of neutrophils result in substantially complete digestion of cellular DNA without significant loss of nuclear or cellular morphology. The control is the result of repeating the inventive method on a

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sample from the same source, except that the neutrophils are
not subjected to DNase treatment.

5   The present invention also provides kits containing
reagents useful for identifying the presence or absence of
p-ANCA of UC, PSC or AIH in a sample. The kits include,
among other reagents, fixed neutrophil and a detectable
secondary antibody.

DETAILED DESCRIPTION OF THE INVENTION

10   The present invention provides methods and kits for
detecting the presence of perinuclear anti-neutrophil
cytoplasmic autoantibody (p-ANCA) for ulcerative colitis
(UC), primary sclerosing cholangitis (PSC) or type 1
autoimmune hepatitis (Type 1 AIH) in a sample. Inventive
methods involve assaying for the loss of a positive value
15   (as compared to a control) upon treatment of neutrophils
with DNase. The inventive methods also involve the detecion
of a particular staining pattern which can be correlated to
the presence of a particular disease associated p-ANCA.

As the name indicates, antibodies to cytoplasmic
20   components of the neutrophil are found in the serum of
patients with certain chronic inflammatory conditions. By
immunofluorescent microscopy, ANCA activity has been divided
into two broad categories: cytoplasmic neutrophil staining
(refered to herein as "c-ANCA staining pattern" or
25   "cytoplasmic staining pattern") and cytoplasmic staining
with perinuclear highlighting (refered to herein as "p-ANCA
staining pattern" or "perinuclear staining pattern").
These distinct staining patterns are obtained with alcohol-
fixed cytocentrifuged neutrophils. It has been reported
30   that the p-ANCA staining pattern is an artifact of alcohol
fixation which results when cytoplasmic granules re-locate
to the periphery of the nucleus during the fixation process.

However, the present invention provides evidence that the perinuclear staining pattern of p-ANCA associated with UC is not artifactual, but rather is the result of specific binding with a DNA associated antigen. Nevertheless, whether alcohol induced or actual, these staining patterns have served to distinguish between types of ANCA arising from unique antigens and having different disease associations.

The methods of the present invention exploit the unique staining patterns of UC, PSC and Type 1 AIH, as compared to one another, CD and other inflammatory conditions of the intestines, to provide a convenient and reliable method of identifying UC, PSC or Type 1 AIH, eliminating the uncertainty formerly associated with diagnosing and treating IBD and these liver diseases.

One aspect of the present invention relates to methods of measuring the presence or absence of p-ANCA of UC or PSC in a sample, comprising: (a) contacting the sample and a detectable secondary antibody with immobilized neutrophils under conditions suitable to form a complex of neutrophil, p-ANCA and detectable secondary antibody, wherein said immobilized neutrophil is subjected to DNase under conditions sufficient to cause substantially complete digestion of cellular DNA without significant loss of nuclear or cellular morphology prior to said contacting step, and wherein said secondary antibody has specificity for p-ANCA or the class determining portion of p-ANCA; (b) separating unbound secondary antibody from the complex; (c) assaying for the presence or absence of p-ANCA containing complex by measuring the presence or absence of bound secondary antibody, compared to a control, wherein said control is the result of repeating the steps of the present method on a sample from the same source, except that the neutrophil of step (a) is not subjected to DNase treatment.

In a related embodiment of the invention, the same
method in an indirect immunofluorescence assay format can be
used to detect the presence or absence of p-ANCA associated
with Type I AIH, as well as the presence or absence of p-
ANCA UC or PSC. Accordingly, there is provided a method of
measuring the presence or absence of p-ANCA associated with
UC, PSC, or Type 1 AIH in a sample, said method comprising:
(a) contacting the sample and a detectable secondary
antibody with fixed neutrophils under conditions suitable to
form an immune complex of neutrophil, p-ANCA and detectable
secondary antibody, wherein said fixed neutrophils are
subjected to DNase under conditions sufficient to cause
substantially complete digestion of cellular DNA without
significant loss of nuclear or cellular morphology prior to
said contacting step, and wherein said secondary antibody
has specificity for the class determining portion of p-ANCA;
(b) separating unbound secondary antibody from the immune
complex; (c) assaying for the pattern of p-ANCA
immunoreactivity by detecting the presence, absence or
pattern of complexed secondary antibody, compared to a
control, wherein said control is the result of repeating the
present method on a sample from the same source, except that
the neutrophils are not subjected to DNase.

As used herein the terms "complex" or "immune complex"
refer to the product of specific bining between an antigenic
determinant-containing molecule, such as an antigen, and a
molecule containing an antibody combining site such as, for
example, an antibody molecule. The term "immunoreactivity,"
as used herein, refers to the ability or attribute of a
molecule containing an antibody combining site, for example
an antibody molecule and the like, to specifically bind an
antigenic determinant-containing molecule such as, for
example, an antigen and the like.

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In the methods of the present invention, neutrophils are subjected to DNase under conditions sufficient to cause substantially complete digestion of cellular DNA. By the term "complete digestion of cellular DNA" it is meant such
5   digestion of the cellular DNA that the cellular DNA has substantially lost its ability to bind proteins and other cellular materials normally associated with the cellular DNA of the neutrophil. Without being bound by any particular theory, it is presently believed that at least part of the
10   antigens of p-ANCA of UC and PSC are proteins that are either intimately associated with nuclear DNA or with some aspects of nuclear structure.

Conditions sufficient to cause substantially complete digestion of cellular DNA will vary in accordance with the
15   purity and concentration of the DNase used and include, for example, incubating the immobilized neutrophil in a concentration of DNase of about 2 to 10 units of DNase per milliliter of a suitable buffer for a time in the range of about 15 minutes to one hour at a temperature in the range
20   of about 22°C to 40°C.

The assays of the present invention may be forward, reverse or simultaneous as described in U. S. Patent No. 4,376,110, issued March 8, 1983 to David et al., incorporated herein by reference in its entirety. In the
25   forward assay, each reagent is sequentially contacted with immobilized neutrophils. If desired, separation of bound from unbound reagent can be accomplished before the addition of the next reagent. In a reverse assay, all reagents are pre-mixed prior to contacting immobilized neutrophil. A
30   modified method of a reverse assay is described in U.S. Patent No. 4,778,751 issued October 18, 1988 to El Shami et al., incorporated herein by reference in its entirety. In a simultaneous assay, all reagents are separately but contemporaneously contacted with the immobilized neutrophil.

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The steps of the presently preferred inventive assay are discussed in further detail below.

As used herein, the term "reagent" refers to any component useful to perform the assays of the present invention, for example, the sample, the primary antibody, the detectible secondary antibody, washing buffers, solutions, and the like.

A sample can be obtained from any biological fluid, for example, whole blood, plasma, or other bodily fluids or tissues having p-ANCA, preferably serum.

The separation steps for the various assay formats described herein, including removing unbound secondary antibody from the complex, can be performed by methods known in the art. When appropriate, a simple washing with a suitable buffer followed by filtration or aspiration is sufficient. If the neutrophils are immobilized on a particulate support, as in the case of microparticles for example, it may be desirable to centrifuge the particulate material, followed by removal of wash liquid. If the neutrophil(s) immobilized on membranes or filters, applying a vacuum or liquid absorbing member to the opposite side of the membrane or filter allows one to draw the wash liquid through the membrane or filter.

The methods of the present invention are normally carried out at room temperature and 37°C. Because the methods involve the use of proteins, temperatures which would substantially modify the tertiary and quaternary structures of the proteins should be avoided. Accordingly, temperatures suitable for performing the methods of the present invention generally range from about 22°C to about 38°C.

In a preferred embodiment of the present invention, neutrophils are are immobilized on a solid substrate. The solid substrate can be any support useful in immunometric

assays. The substrate can be made from natural or synthetic material which is insoluble in water and can be rigid or non-rigid. However, the substrate should not significantly affect the desired activity of the neutrophils. Preferred substrates include glass slides, test wells made from polyethylene, polystyrene, nylon, nitrocellulose, glass and the like. Also useful are test tubes, filter paper, filtering devices such as glass membranes, beads, and particulate materials such as agarose, cross-linked dextran and other polysaccharides, and the like.

In accordance with the methods and kits of the present invention, immobilization of neutrophils can be accomplished by any method known in the art. Preferably, a method of immobilization is used that renders the neutrophils permeable to DNase and the reagents used in the methods and kits of the present invention. For example, neutrophils can be immobilized by fixing them directly to the surface of a test well or glass slide with suitable fixative, such as, for example, methanol, ethanol, formalin, or the like. Of course, one of skill in the art will appreciate that such fixative should not substantially alter nuclear or cellular morphology of the neutrophils.

Neutrophils and secondary antibody appropriate for use in the practice of the present invention will depend upon the origin of the sample assayed. As used herein, the terms "patient," "subject," or "individual" when referring to the origin of the sample to be assayed, means any animal capable of producing p-ANCA of UC, PSC or Type 1 AIH, including for example, humans, non-human primates, rabbits, rats, mice, and the like. Preferably, neutrophils and secondary antibody employed will have specific reactivity for the species from which the sample to be tested is obtained. For example, to assay for p-ANCA of UC, PSC, or Type I AIH in a sample obtained from a human subject, the neutrophils and

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the secondary antibody are preferably specific for humans.
If multiple antibodies are employed, each antibody is
preferably species-specific for its antigen.

Neutrophils useful in the present invention can be
obtained from a variety of sources, e.g., the blood of a
human, non-human primates, rabbits, rats, mice, and the
like, by methods known to those of skill in the art.

The term "secondary antibody" as used herein, refers to
any antibody or combination of antibodies or fragments
thereof, at least one of which can bind p-ANCA of UC, PSC,
or Type I AIH. For example, a secondary antibody can be an
anti-p-ANCA antibody, specific for any epitope of p-ANCA,
but preferably not one that would be competitive with
neutrophil binding or cause steric hinderance of
neutrophil/p-ANCA binding. Alternatively, a secondary
antibody can be an anti-IgG preferably having specificity
for the class determining portion of p-ANCA.

Secondary antibodies useful in the practice of the
present invention can be obtained by techniques well known
in the art. Such antibodies can be polyclonal or preferably
monoclonal. Polyclonal antibodies can be obtained, for
example, by the methods in Ghose et al., Methods of
Enzymology, Vol. 93, 326-327 (1983). For example, IgG or Fc
fragments of IgG can be used as the immunogen to stimulate
the production of IgG reactive polyclonal antibodies in the
antisera of animals such as rabbits, goats, sheep, rodents,
and the like.

Monoclonal antibodies useful in the practice of the
present invention can be obtained from a number of
commercially available sources. Alternatively, the
antibodies can be obtained, for example, by the process
described by Milstein and Kohler in Nature, 256:495-97
(1975) or as modified by Gerhard, Monoclonal Antibodies,
370-371 (Plenum Press, 1980). If a mouse anti-human IgG

antibody is desired, a mouse is first injected with an immunogen containing, for example, human IgG or Fc fragments of human IgG. The mouse is subsequently sacrificed and cells taken from its spleen are fused with myeloma cells by methods well known in the art. The resulting hybridomas are screened to isolate clones that secrete a single antibody specie reactive with human IgG.

Preferably, the hybridomas are screened to identify those producing antibodies that are highly specific for the IgG of interest. The monoclonal antibody selected will have an affinity compatible with the desired sensitivity and range for detecting p-ANCA of UC or PSC. The use of such monoclonal antibodies provides a means of obtaining greater sensitivity in the assays of the present invention compared with the use of polyclonal antibodies.

Alternatively, monoclonal antibodies having a high affinity for p-ANCA of UC or PSC can be obtained by the creation of a phage combinatorial library for p-ANCA of UC or PSC and then screening for specificity by a similar process described in Barbas, C.F. et al, Proceedings of the Nat'l Academy of Science, 88:7978-82 (1991), incorporated herein by reference. The Examples below exemplify methods for making a phage combinatorialy library of an immunoglobulin gene repertoire for UC, as well as methods of screening the library for p-ANCA associated with UC. The nucleic acid and deduced amino acid sequence of the immunoglobulin heavy and light Fab chains of the two clones (5-3 and 5-4) of p-ANCA associated with UC are provided in SEQ ID NO. 1 through 8. Anti-idiotypic antibodies to these and other clones of p-ANCA associated with UC can be raised by methods well known in the art. For example, polyclonal and monoclonal antibodies can be produced as described, for example, in Harlow and Lane, Antibodies: A Laboratory

Manual (Cold Spring Harbor Laboratory 1988), which is incorporated herein by reference.

The phrase "monoclonal antibody" in its various grammatical forms refers to a population of antibody
5 molecules that contain only one species of idiotope capable of immunoreacting with a particular epitope on an antigen or idiotope on an antibody. A monoclonal antibody typically displays a single binding affinity for an epitope or idiotope with which it immunoreacts; however, a monoclonal
10 antibody may be a molecule having a plurality of idiotopes, each immunospecific for a different epitope or idiotope, e.g., a bispecific monoclonal antibody.

Monoclonal antibodies are typically composed of antibodies produced by clones of a single cell called a
15 hybridoma that secretes (produces) but one kind of antibody molecule. In accordance with the present invention hybridomas capable of producing antibody material having specific immunoreactivity with p-ANCA associated with UC, but which does not prevent immunoreactivity of p-ANCA with
20 neutrophil is provided. Such a hybridoma cell is formed by fusing an antibody-producing cell and a myeloma or other self-perpetuating cell line. The preparation of such hybridomas was first described by Kohler and Milstein, Nature, 256:495-497 (1975), which description is
25 incorporated by reference. Polypeptide-induced hybridoma technology is also described by Niman et al., Proc. Natl. Sci. U.S.A., 80:4949-4953 (1983), which description is also incorporated herein by reference.

To obtain an antibody-producing cell for fusion with an
30 immortalized cell, a mammal is inoculated with an immunogen. The word "immunogen" in its various grammatical forms is used herein to describe a composition containing a p-ANCA associated with UC as an active ingredient used for the

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preparation of the antibodies against p-ANCA associated with UC.

The amount of p-ANCA associated with UC used to inoculate the mammal should be sufficient to induce an immune response to the immunizing polypeptide. This amount depends, among other things, on the species of animal inoculated, the body weight of the animal and the chosen inoculation regimen as is well known in the art. Inocula typically contain about 10 micrograms of immunogen per inoculation for mice and may contain up to about 500 milligrams of immunogen per inoculation for larger mammals.

The spleen cells of the mammal immunized with p-ANCA associated with UC are then harvested and can be fused with myeloma cells using polyethylene glycol (PEG) 1500. Fused hybrids are selected by their sensitivity to HAT. Hybridomas producing an anti-p-ANCA idiotypic monoclonal antibody can be identified by screening hybridoma supernatants for the presence of antibody molecules that immunoreact with p-ANCA associated with UC. Such screening methods include for example, radioimmunoassay (RIA) or enzyme linked immunosorbent assay (ELISA).

Media useful for the preparation of these compositions are well known in the art and commercially available and include synthetic culture media, inbred mice and the like. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; Dulbecco et al., _Virol._, 8:396 (1959)) supplemented with 4.5 gm/l glucose, 20 mm glutamine, and 20% fetal calf serum. An exemplary inbred mouse strain is the Balb/c.

Another alternative for increasing the sensitivity of the assay of the present invention is to use a multiple antibody system for the secondary antibody, rather than using a single antibody with enhanced specificity. Thus,

the methods of the present invention may be performed using a combination of antibodies as the secondary antibody, wherein at least one secondary antibody of the combination has specificity for p-ANCA or the class determining portion of p-ANCA and at least one secondary antibody of the combination is detectable. For example, UC and PSC may be distinguished from Crohn's disease in a sample of human blood by contacting two aliquots of blood serum from a patient with immobilized untreated or DNase treated human neutrophil, followed by contacting the resulting antibody-antigen complex with mouse anti-human IgG. The resulting complex is then contacted with goat anti-mouse IgG having a detectable label and washed to remove unbound antibody. The resulting complex is assayed for the presence or absence of a detectable complex, compared to the control (i.e., non-DNase treated neutrophil). The absence of the labeled goat anti-mouse IgG complexed with DNase-treated neutrophils indicates that the patient has UC or PSC.

The term "detectible secondary antibody" refers to secondary antibody, as defined above, that can bind p-ANCA of UC or PSC and can be detected or measured by a variety of analytical methods. This term includes antibodies, or fragments thereof, that are directly detectible without attachment of signal generating labels, or those that can be labeled with a signal generating system to permit detection or measurement, such as, for example, any secondary antibody capable of being labeled with a radioisotope, enzyme, chromogenic or fluorogenic substance, a chemiluminescent marker, or the like. Alternatively, a secondary antibody can be made detectible by using biotin-avidin linkage to associate a label with the secondary antibody. In any of the above methods, the reactivity of the secondary antibody with the p-ANCA should not be significantly altered by the presence of the label. When a multi-antibody system is used

as the secondary antibody, at least one of the antibodies, combination of antibodies or fragments thereof is capable of binding p-ANCA of UC or PSC, and at least one can readily be detected or measured by suitable analytical methods.

5   Detectible markers can be bound to the secondary antibody by procedures known to those skilled in the art such as, for example, the chloramine-T procedure for radioactive markers, enzymatically by the lactoperoxidase procedure, by the Bolton-Hunter techniques or any other
10   technique known in the art. These techniques plus others are well known to those of skill in the art and are described, for example, in Methods in Enzymology, Volume 70, Part A (Van Vunakis and Langone, editors 1980).

          Thus, the secondary antibody can be bound to enzymes
15   such as, for example, horseradish peroxidase, luciferase, malate dehydrogenase, glucose-6-phosphate dehydrogenase, alkaline phosphatase, and the like. The presently preferred enzyme is alkaline phosphatase. Dual channeled catalytic systems may also be used in the methods of the present
20   invention, including, for example, alkaline phosphatase and glucose oxidase using glucose-6-phosphate as the initial substrate. Suitable catalytic systems are described in U.S. Patent No. 4,366,241, issued December 28, 1982 to Tom et al., U.S. Patent No. 4,740,468, issued April 26, 1988 to
25   Weng et al., U.S. Patent No. 4,843,000, issued June 27, 1989 to Litman et al., and U.S. Patent No. 4,849,338, issued July 18, 1989 to Litman et al., all of which are herein incorporated by reference in their entirety.

          The procedures for attaching enzymes to various
30   substances are well known in the art. For example, techniques for coupling enzymes to antibodies are described in J.H. Kennedy et al., Clin. Chim. Acta, 70:1 (1976). Reagents useful for such coupling include, for example, glutaraldehyde, p-toluene diisocyanate, various carbodiimide

reagents, p-benzoquinone m-periodate, N,N'-ortho-
phenylenedimaleimide, and the like.

Alternatively, secondary antibody linked to a
detectable enzyme useful for the methods and kits of the
present invention may be obtained from a number of
commercially available sources, for example, goat F(ab')2
anti-human IgG-alkaline phosphatase may be purchased from
Jackson Immuno-Research, located in West Grove,
Pennsylvania.

Suitable substrates for the above-described enzymatic
systems include simple chromogens and fluorogens such as,
for example, beta-D-glucose, homovanillic acid, o-
dianisidine, bromocresol purple powder, 4-methyl-
umbelliferone, luminol, para-dimethylaminolophine,
paramethoxylophine, para-nitrophenyl phosphate, and the
like. The presently preferred enzyme substrate is para-
nitrophenylphosphate.

Secondary antibody may also be rendered detectable by
chemically linking it to a fluorogenic compound. Suitable
fluorogenic compounds are those that emit light in
ultraviolet or visible wavelength subsequent to excitation
by light or other energy source. The fluorogens can be
employed alone or with a suitable quencher molecule.
Presently preferred fluorogens are fluorescein, fluorescein
isothiocyanate, tetramethyl-rhodamine isothiocynate, 7-
amino-4-methylcoumarin-3-acetic acid and phycoerythrin. The
methods of conjugating and using these and other suitable
fluorogens have been reported and are described, for
example, in Methods in Enzymology, Volume 74, Part C, 3210S
(Van Vunakis and Langone, Editors 1991).

Alternatively, secondary antibody linked to fluorogen
useful for the practice of the present invention may be
obtained from a number of commercially available sources,

for example, goat F(ab')2 anti-human IgG-FITC available from
Tago Immunologicals, Burlingame, California.

Depending on the nature of the label or catalytic
signal producing system used, a signal can be detected by
5   irradiating the complexed test sample with light and
observing the level of fluorescence; by contacting the
complexed sample with a substrate which can be catalytically
converted by the label to produce a dye, fluorescence or
chemiluminescence, in which the formation of dye can be
10   observed visually or in a spectrophotometer; fluorescence
can be observed visually or in a fluorometer; or, in the
case of chemiluminescence or a radioactive label, by
employing a radiation counter such as a gamma counter or
gamma emitting markers such as iodine-125. For enzyme-
15   catalyzed systems, when the presently preferred combination
of alkaline phosphatase is used as the enzyme and para-
nitrophenyl phosphate as the substrate, a color change may
be detected visually for a qualitative positive reaction.
For a quantitative analysis of the same or similar system,
20   EMAX Microplate Reader (available from Molecular Devices,
Menlo Park, California) at 405 nm may be used in accordance
with the manufacturer's instructions.

In accordance with the present invention, the presence
or absence of p-ANCA of UC or PSC in the sample being tested
25   is determined by contacting a sample with immobilized, DNase
treated neutrophils and secondary antibody, and assaying for
the presence or absence of p-ANCA containing complex. The
presence or absence of p-ANCA containing complex is
determined by monitoring for the presence or absence of
30   bound secondary antibody, compared to a control. P-ANCA is
considered present in the test sample if there exists a loss
of positive value (bound secondary antibody) in the test
sample as compared to the control. The control is the
result of repeating the same steps of the inventive method

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on a sample from the same source, when the immobilized
neutrophil has not been subjected to DNase.

For example, in an IIF assay format of the present
methods the presence of p-ANCA of UC in a sample, and thus
5   UC itself, is indicated when there is a loss of a
perinuclear staining pattern, i.e., detectable complex
associated with perinuclear staining pattern, as compared to
said control. More preferably, the presence of p-ANCA of UC
is further indicated by the absence of both a perinuclear
10   staining pattern and a cytoplasmic staining pattern in the
sample. Similarly, using the same IIF assay format the
presence of p-ANCA of PSC in a sample, and thus PSC itself,
is indicated when a homogeneous cytoplasmic staining pattern
is detected in the sample and a perinuclear staining pattern
15   is detected in the control, i.e., "conversion of detectable
complex associated with perinuclear staining pattern to
homogenous cytoplasmic staining pattern, as compared to said
control." Likewise, using the same IIF assay format, the
presence of p-ANCA of Type 1 AIH in a sample, and thus Type
20   1 AIH itself, is indicated when a granular cytoplasmic
staining pattern is detected in the sample and a perinuclear
staining pattern is detected in the control, i.e.,
"conversion of detectable complex associated with
perinuclear staining pattern to granular cytoplasmic
25   staining pattern, as compared to said control." Finally, CD
is indicated if the absence of a perinuclear staining
pattern in the control is detected, i.e., "absence of a
detectable complex associated with perinuclear staining
pattern in said control."

30   In this manner, the methods of the present invention
can be used to distinguish between p-ANCA of UC, p-ANCA of
PSC and p-ANCA of Type 1 AIH, as well as to screen for any
one of these p-ANCA, and thereby, preferably in combination

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with traditional diagnostic techniques, screen for any one
of the diseases and distinguish them from CD.

For example, sera from 94 patients diagnosed with UC
which were seropositive for p-ANCA, sera from ten patients
5   diagnosed with PSC which were seropositive for p-ANCA, and
sera from 22 patients diagnosed with Type I AIH which were
seropositive for very high titre p-ANCA (mean ELISA value
for neutrophil binding 139±8) were analyzed for DNase
sensativity in accordance with the methods of the present
10 invention using a IIF assay format. As summarized in
Table 1, loss of antigenic recognition after DNase digestion
of neutrophils demonstarted by the absence of any staining
pattern is a dominant (66/94, 70%) characteristic of p-ANCA
associated with UC.

15 Table 1. Reactions of p-ANCA expressing sera with DNase
          treated neutrophils

		No Treatment	Post DNase Treatment	
			Neutrophil Staining Pattern	
Disease	n	perinuclear	None	cANCA
UC	94	94/94 (100%)	66/94 (70%)	28/94 (30%)
Type 1 AIH	22	22/22 (100%)	3/22 (14%)	19/22 (86%)
PSC (w/o UC)	10	10/10 (100%)	3/10 (30%)	7/10 (70%)

On the other hand, the majority of p-ANCA associated with
PSC and p-ANCA associated with Type 1 AIH p-ANCA recognize
cytoplasmic components after DNase treatment of neutrophils
(7/10, 70% and 19/22, 86% respectively). When the patient
25 sera is grouped based on whether the patient had UC or not
(Table 2, UC/non-UC), it becomes clear that loss of
perinuclear staining pattern after DNase treatment of

neutrophil is unique to p-ANCA of UC providing a reliable
basis on which to screen for UC and differentiate p-ANCAs.

Table 2.   Comparison of p-ANCA of UC and group of p-ANCA
           associated with diseases other than UC.

| | | No Treatment | Post DNase Treatment | |
| | | | Neutrophil Staining Pattern | |
Disease	n	p-ANCA	None	cANCA
UC	94	94/94 (100%)	66/94 (70%)	28/94 (30%)
non-UC	32	32/32 (100%)	6/32 (19%)	26/32 (81%)

The differentiation between p-ANCA of PSC and p-ANCA of
Type 1 AIH is based upon the specific cytoplasmic staining
pattern produced with DNase treated neutrophil. As
exemplified in by the drawing, the perinuclear staining
pattern of p-ANCA positive PSC sera (Fig. 1B) in the
majority of sera tested became cytoplasmic but with a
characteristically mushy, or stated more scientifically,
homogenous staining pattern (Fig. 1D). By comparison, the
perinuclear staining pattern generated by Type 1 AIH serum
with methanol-fixed neutrophils (Fig. 1A) in the majority of
sera tested also became cytoplasmic, but with a
characteristically granular staining pattern (Fig. 1C).

Accordingly, another embodiment of the invention
provides methods of detecting the presence or absence of p-
ANCA associated with Type 1 AIH in a sample, comprising:
(a) contacting fixed neutrophils with a sample and a
detectable secondary antibody under conditions suitable to
form an immune complex of neutrophil, p-ANCA and detectable
secondary antibody, wherein cellular DNA of the fixed
neutrophils has been digested by DNase without significant
loss of nuclear or cellular morphology, and wherein the
detectable secondary antibody is detectable by fluorescence

and is specific for the class determining portion of p-ANCA;
(b) separating unbound secondary antibody from the immune
complex; and (c) detecting the immunofluorescent staining
pattern of the complex as compared to a control, wherein the
control is the result of repeating the present method using
fixed neutrophils wherein the cellular DNA of the fixed
neutrophils has not been digested by DNase, and wherein the
presence of a granular cytoplasmic staining pattern in the
sample, and a perinuclear staining pattern in the control,
indicates the presence of p-ANCA associated with type 1
autoimmune hepatitis in the sample. The skilled artisan
will appreciate that the control as described above, is
generated using neutrophil which has been fixed in the same
manner as the neutrophil used to test the sample, but that
the neutrophils used to generate the control have not been
subjected to treatment i.e., digestion, with DNase.

In accordance with another embodiment of the present
invention, there is provided methods of detecting the
presence or absence of p-ANCA associated with PSC in a
sample, comprising: (a) contacting fixed neutrophils with a
sample and a detectable secondary antibody under conditions
suitable to form an immune complex of neutrophil, p-ANCA and
detectable secondary antibody, wherein cellular DNA of the
fixed neutrophils has been digested by DNase without
significant loss of nuclear or cellular morphology, and
wherein the detectable secondary antibody is detectable by
fluorescence and is specific for the class determining
portion of p-ANCA; (b) separating unbound secondary antibody
from the immune complex; and (c) detecting the
immunofluorescent staining pattern of the complex as
compared to a control, wherein the control is the result of
repeating the present method using fixed neutrophils wherein
the cellular DNA of the fixed neutrophils has not been
digested by DNase, and wherein the presence of a homogenous

cytoplasmic staining pattern in the sample, and a
perinuclear staining pattern in the control, indicates the
presence of p-ANCA associated with primary sclerosing
cholangitis in the sample.

5   In yet another embodiment of the present invention
there is provided, methods of differentiating p-ANCA of PSC
from p-ANCA of Type I AIH, and thus differentiating between
the presence of the diseases, comprising: (a) contacting
fixed neutrophils with a sample and a detectable secondary

10   antibody under conditions suitable to form an immune complex
of neutrophil, p-ANCA and detectable secondary antibody,
wherein cellular DNA of the fixed neutrophils has been
digested by DNase without significant loss of nuclear or
cellular morphology, and wherein the detectable secondary

15   antibody is detectable by fluorescence and is specific for
the class determining portion of p-ANCA; (b) separating
unbound secondary antibody from the immune complex; and (c)
detecting the immunofluorescent staining pattern of the
complex as compared to a control, wherein the control is the

20   result of repeating the present method using fixed
neutrophils wherein the cellular DNA of the fixed
neutrophils has not been digested by DNase, and wherein the
presence of a homogeneous cytoplasmic staining pattern in
the sample and a perinuclear staining pattern in the control

25   indicates PSC, and wherein the presence of a granular
cytoplasmic staining pattern in the sample and a perinuclear
staining pattern in the control indicates Type I AIH.

          In yet another embodiment of the present invention
there is provided, methods of differentiating p-ANCA of UC

30   from p-ANCA of Type I AIH, and thus differentiating between
the presence of the diseases, comprising: (a) contacting
fixed neutrophils with a sample and a detectable secondary
antibody under conditions suitable to form an immune complex
of neutrophil, p-ANCA and detectable secondary antibody,

wherein cellular DNA of the fixed neutrophils has been digested by DNase without significant loss of nuclear or cellular morphology, and wherein the detectable secondary antibody is detectable by fluorescence and is specific for the class determining portion of p-ANCA; (b) separating unbound secondary antibody from the immune complex; and (c) detecting the immunofluorescent staining pattern of the complex as compared to a control, wherein the control is the result of repeating the present method using fixed neutrophils wherein the cellular DNA of the fixed neutrophils has not been digested by DNase, and wherein the absence of a perinuclear staining pattern in the sample, and preferably the absence of a cytoplasmic staining pattern in the sample as well, and a perinuclear staining pattern in the control sample indicates UC, and wherein the presence of a granular cytoplasmic staining pattern in the sample and a perinuclear staining pattern in the control indicates Type I AIH.

In still another embodiment of the present invention there is provided methods of differentiating between p-ANCA of UC, p-ANCA of PSC, and p-ANCA of Type 1 AIH, and thus differentiating between the presence of the diseases, said method comprising: (a) contacting fixed neutrophils with a sample and a detectable secondary antibody under conditions suitable to form an immune complex of neutrophil, p-ANCA and detectable secondary antibody, wherein cellular DNA of the fixed neutrophils has been digested by DNase without significant loss of nuclear or cellular morphology, and wherein the detectable secondary antibody is detectable by fluorescence and is specific for the class determining portion of p-ANCA; (b) separating unbound secondary antibody from the immune complex; and (c) detecting the immunofluorescent staining pattern of the complex as compared to a control, wherein the control is the result of

repeating the present method using fixed neutrophils wherein the cellular DNA of the fixed neutrophils has not been digested by DNase, and wherein absence of a perinuclear staining pattern in the sample, and preferably the absence of a cytoplasmic staining pattern in the sample as well, and the presence of a perinuclear staining pattern in the control indicates UC; wherein the conversion of detectable complex associated with perinuclear staining pattern to homogenous cytoplasmic staining pattern, as compared to said control, indicates PSC; wherein conversion of detectable complex associated with perinuclear staining pattern to granular cytoplasmic staining pattern, as compared to said control, indicates Type I AIH; and wherein absence of a detectable complex associated with perinuclear staining pattern in said control indicates CD.

In another aspect of the present invention, kits for measuring the presence of the p-ANCA of UC, PSC, or Type 1 AIH in a sample are provided. A kit of the present invention can contain immobilized, DNase-treated, alcohol fixed neutrophils and a detectable secondary antibody. Alternatively, a kit may contain immobilized neutrophils, DNase and a detectable secondary antibody. Optionally, depending on the secondary antibody or label used, the kits may contain a signal generating substance to provide or enhance the detection of the p-ANCA of UC, PSC or Type 1 AIH. In addition, other components such as ancillary reagents may be included, for example, stabilizers, buffers, fixatives, such as methanol or ethanol, and the like. The reagents can be provided as dry powders, usually lyophilized, including excipients, which on dissolution will provide a reagent solution having the appropriate concentrations for performing the methods of the present invention.

A preferred embodiment of the inventive kit includes DNase and human neutrophils immobilized on a solid substrate, preferably a microtiter plate or beads for detecting or quantitating fluorescence by a cell sorter. To detect the presence of p-ANCA of UC, PSC or Type 1 AIH, the kit preferably includes mouse anti-human IgG, and goat anti-mouse IgG labeled with an enzyme or a fluorogenic substance.

In yet another aspect of the present invention there is provided an isolated antigen of UC. The antigen naturally occurs in neutrophils and is characterized by its insolubility in Triton X-100™ which can be obtained from Fisher, Pittsburgh, Pennsylvania, catalogue number BP-151.

In yet another aspect of the present invention there is provided an isolated antigen of PSC. The antigen naturally occurs in neutrophils and is characterized by its insolubility in Triton X-100™.

The invention will now be described in greater detail by reference to the following non-limiting examples.

EXAMPLE I

SEPARATION OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES BY FICOLL-HYPAQUE GRADIENT CENTRIFUGATION

1. Add 31.8 g Ficoll 400 (Pharmacia, Sweden) to 400 ml deionized H_2O in a 500 ml bottle. Shake vigorously until dissolved. Add 100 ml of 50% sodium diatrizoate hypaque (UCLA Pharmacy, Los Angeles, California) and mix.

2. Check specific gravity using a hydrometer. It should be 1.077-1.080.

3. Filter-sterilize Ficoll-hypaque solution through a 0.22 or 0.45 um bottle top filter. The Ficoll-hypaque solution may be stored at 4°C, protected from light.

4. Pour 15 ml Ficoll-hypaque solution into a 50 ml conical centrifuge tube. Carefully overlayer 30 ml heparinized blood.

5. Centrifuge at 1000 x g (2000 RPM) for 20 min.

6. Remove interface using a serologic pipet or pasteur pipet and place into 50 ml conical centrifuge tube.

7. Dilute interface layer with at least an equal volume of Hanks' Balanced Salt Solution (HBSS) (Irvine Scientific, Santa Ana, California).

8. Centrifuge at 400 x g (1200 RPM) for 5 min.

9. Decant supernatant, resuspend pellet, and add 50 ml HBSS.

10. Repeat twice steps 8 and 9.

11. Resuspend cells in RPMI 1640 (Irvine Scientific, Santa Ana, California) + 5% fetal calf serum (GIBCO, Gathersberg, Maryland).

EXAMPLE II

ISOLATION OF NEUTROPHIL

1. Using a pipet, carefully remove serum and remaining Ficoll-Hypaque from red blood cell pellet resulting from procedure described in Example I.

2. Add 10 ml 6% dextran to 15 milliliters of pellet.

3. Top off with 1X HBSS to 50 ml. Re-suspend pellet.

4. Allow red blood cells to settle, approximately 45 minutes to one hour.

5. Separate supernatant, discard pellet. Top supernatant off with 1X HBSS to 50 ml and centrifuge for 5 minutes at 1800 rpm.

6. Decant supernatant and tap pellet. Hypotonically lyse remaining red blood cells by adding 9 ml

deionized water, swirl, and then add 1 ml 10X HBSS
and immediately dilute with 1X HBSS to 50 ml.

    7.   Centrifuge for 5 minutes at 1000 rpm. Discard
supernatant and re-suspend pellet in 15 ml 1X
HBSS.

5

EXAMPLE III
IMMOBILIZATION OF Neutrophils ON GLASS SLIDES

    1.   Count cells in suspension of step 7 of Example II
using a microscope and hemacytometer and re-
suspend cells in sufficient volume of 1X HBSS to
achieve $2.5X10^6$ cells per ml.

10

    2.   Use Cytospin 3™ (Shandon, Inc. Pittsburgh,
Pennsylvania) at 500 rpm for 5 minutes to apply
0.01 ml of the re-suspended cells to each slide.

    3.   Fix cells to slide by incubating slides for 10
minutes in sufficient volume of 100% methanol to
cover sample. Allow to air dry. The slides may
be stored at -20°C.

15

EXAMPLE IV
DNase TREATMENT OF Neutrophils IMMOBILIZED ON GLASS SLIDE

20

    Prepare a DNase solution by combining 3 units of
Promega RQ1™ DNase per ml buffer containing 40 mM of TRIS-
HCl (pH 7.9), 10 mM of sodium chloride, 6 mM magnesium
chloride and 10 mM calcium chloride. Promega RQ1™ DNase can
be obtained from Promega, of Madison, Wisconsin.

25

    Rinse slides prepared in accordance with Example III
with about 100 ml phosphate buffered saline (pH 7.0-7.4) for
5 minutes. Incubate immobilized neutrophils in 0.05 ml of
DNase solution per slide for about 30 minutes at 37°C. Wash

the slides three times with about 100-250 ml phosphate
buffered saline at room temperature.

EXAMPLE V

IMMUNOFLUORESCENCE ASSAY

5   1.   Add 0.05 ml of a 1:20 dilution of human sera in
           phosphate buffered saline to slides treated with
           DNase in accordance with Example IV and to
           untreated slides of Example III. Add 0.05 ml
           phosphate buffered saline to clean slides as
10   blanks. Incubate for 0.5 to 1.0 hours at room
           temperature in sufficient humidity to minimize
           volume loss.

     2.   Rinse off sera by dipping into a container having
           100-250 ml phosphate buffered saline. Soak slide
15   in phosphate buffered saline for 5 minutes. Blot
           lightly.

     3.   Add 0.05 ml goat F(ab')$_2$ anti-human IgG(μ)-FITC,
           at a 1:1000 antibody:phosphate buffered saline
           dilution, to each slide. Incubate for 30 minutes
20   at room temperature, in sufficient humidity to
           minimize volume loss. (Goat F(ab')$_2$ anti-human
           IgG(μ)-FITC is available from Tago Immunologicals,
           Burlingame, CA and from Jackson Immunoresearch
           Laboratories, Baltimore, MD).

25   4.   Rinse off antibody with 100-250 ml phosphate
           buffered saline. Soak slides for 5 minutes in
           100-250 ml phosphate buffered saline, then allow
           to air dry.

     5.   Read fluorescence pattern on fluorescence
30   microscope at 40X.

30

If desired, any DNA can be stained with propidium iodide stain by rinsing slides well with phosphate buffered saline at room temperature and stain for 10 seconds at room temperature. Wash slide three times with 100-250 ml
5   phosphate buffered saline at room temperature and mount cover slip.

EXAMPLE VI
DNase SENSITIVITY OF UC p-ANCA SPECIFIC ANTIGEN USING IMMUNOFLUORESCENCE ASSAY

10   DNase obtained from Promega was used at a working concentration of 3 units/ml. DNase concentration was optimized by titrating the amount of DNase added (from 1 to 10 units/ml) and examining the extent of DNA digestion by propidium iodide staining and/or reaction with anti-DNA
15   antisera. Digestion of cytocentrifuged, methanol-fixed neutrophils was carried out at 37°C for 30 minutes with DNase solubilized in 40 mM Tris-HCl (pH 7.9) buffer containing 10 mM NaCl, 6 mM MgCl$_2$ and 10 mM CaCl$_2$. Virtually all cellular DNA was lost, as indicated by the lack of
20   propidium iodide staining. Also lost was the reaction of an anti-histone positive serum. DNase reaction carried out as described herein, however, does not significantly alter nuclear or cellular morphology.

Neutrophils treated with trypsin at various
25   concentrations no longer reacted with UC p-ANCA positive sera nor with anti-histone positive serum, indicating that at least part of the p-ANCA reactive antigen is a protein. Similarly, pepsin digestion of neutrophils abolished PSC p-ANCA positive serum reaction, also indicating a
30   proteinaceous character of that antigenic species. Panels of UC p-ANCA positive and c-ANCA positive patient sera were examined for DNase sensitivity using

cytocentrifuged, methanol-fixed slides as described above.
Two other types of reactions were noted. Some p-ANCA
positive sera lost the perinuclear aspect of the reaction
and became cytoplasmic after DNase treatment, while c-ANCA
5   positive sera generally remained cytoplasmic. Additionally,
some sera that were found to have both a perinuclear and
cytoplasmic ANCA staining reaction always lost the
perinuclear aspect of the reaction after DNase treatment of
neutrophils. These DNase-induced staining patterns proved
10   to be highly reproducible from experiment to experiment.

This data indicates at least three ANCA reactions are
possible in response to DNase treatment of immobilized
neutrophils; 1) a p-ANCA reaction that is abolished, 2) a p-
ANCA reaction that becomes cytoplasmic and 3) a c-ANCA
15   reaction that persists. In all of these cases, the DNase
digestion was complete as evidenced by a lack of propidium
iodide staining as well as lack of reaction by anti-DNA
antibody.

To determine whether DNase treatment of neutrophils
20   would abolish the antigenic recognition of all p-ANCA
associated with UC, a panel (n=94) of UC patient sera,
previously characterized as containing p-ANCA was examined
for neutrophil binding after DNase treatment using the IIF
assay format. In 70% of the UC sera tested, DNase treatment
25   again resulted in the abolition of the immunogenic reaction
that results in a p-ANCA staining pattern (Figure 2A and C).
The remaining p-ANCA positive UC sera were found to give a
cytoplasmic (c-ANCA) homogeneous (or mushy) staining pattern
after DNase treatment of neutrophils (Figure 2B and D).
30   Thus, p-ANCA associated with UC yielded two possible
reactions after DNase treatment of neutrophils; 1) a p-ANCA
reaction that is abolished and 2) a p-ANCA reaction that
converts to a c-ANCA staining pattern. These changes in
neutrophil staining patterns obtained after DNase treatment

of cells were a consistent feature of the sera tested and
the same results were obtained in multiple experiments.

Finally, it was also examined whether prior reaction of
neutrophils with p-ANCA positive serum would effect the
5   DNase sensitivity of antigen. The perinuclear reaction is
maintained even after DNase digestion when neutrophils are
first treated with the p-ANCA positive serum. This result
indicates a protective effect of antibody binding against
either physical loss of antigen or loss of epitope
10 recognition.

EXAMPLE VII

COMPARATIVE CHARACTERIZATION OF UC p-ANCA IMMUNOREACTIVE

To examine whether DNA integrity was necessary for UC
p-ANCA binding to neutrophils, methanol-fixed neutrophils
15 were treated with DNase, contacted with p-ANCA positive
serum from a patient diagnosed with UC, and UC specific p-
ANCA binding examined by IIF. For comparative purposes the
binding of a non-UC sera were also tested. Serum that
expresses anti-DNA antibodies (Rheumatology Diagnostics
20 Laboratories Inc., Los Angeles, CA), a serum that expressed
WG ANCA, a serum that expresses anti-elastase antibodies,
and that serum expresses antibodies to PR3 (the latter three
all obtained from J. Charles Jennette University of North
Carolina, Chapel Hill) were also contacted with DNase
25 treated, methanol-fixed neutrophils and binding examined by
IIF. Additionally, the effectiveness of the DNase digestion
and subsequent loss of DNA was routinely monitored by
staining neutrophils with the DNA binding dye, propidium
iodide.

30   Figure 3 and 4 provide for comparison the IIF staining
patterns generated with these serum with methonal-fixed
neutrophil (top row) and DNase-treated, methanol-fixed

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neutrophil (bottom row). As seen in Figure 3A and D, the p-ANCA staining pattern generated by p-ANCA positive UC serum (Fig. 3A) is completely lost when neutrophil are pre-treated with DNase (Fig. 3D) indicating that UC p-ANCA binding is

5   abolished. A similar loss of antigen recognition after DNase treatment was obtained, as expected, with the anti-DNA serum. Figure 3B depicts the IIF staining pattern of anti-DNA serum on untreated neutrophils. This staining pattern is clearly lost when neutrophils are pre-treated with DNase.

10   (Fig. 3E) That DNase treatment of neutrophils was effective in eliminating cellular DNA is seen in the lack of propidium iodide staining after such treatment (Fig. 3F) as compared to the propidium iodide staining pattern in the absence of DNase treatment. (Fig. 3C) Neutrophil binding by WG serum

15   was unchanged (Fig. 4A and D) by DNase treatment of the cells while the anti-elastase p-ANCA staining pattern (Fig. 4B) was converted to a granular cytoplasmic pattern (Fig. 4E) by DNase treatment. Finally, the staining pattern generated by anti-PR3 (Fig. 4C and F) was also unaffected by

20   the DNase digestion of neutrophils.

EXAMPLE VIII

COMPARATIVE DNase SENSITIVITY OF
PSC p-ANCA SPECIFIC ANTIGEN AND TYPE 1 AIH SPECIFIC ANTIGEN
USING IMMUNOFLUORESCENCE ASSAY

25   A panel of p-ANCA-containing sera from PSC and Type 1 AIH patients was examined and compared to the UC sera panel. All the sera were previously characterized with respect to ANCA staining pattern by IIF and ANCA binding level as determined by ELISA. Representative ANCA staining patterns

30   before and after DNase digestion of neutrophils are given in Figure 1.

34

The p-ANCA staining pattern generated by Type 1 AIH serum with methanol-fixed neutrophils is depicted in Figure 1A. This p-ANCA positive Type 1 AIH serum was characteristically found to yield a granular cytoplasmic staining pattern with DNase digested neutrophils. (Fig. 1C) The p-ANCA staining pattern generated by serum of patients diagnosed with PSC is depicted in Figure 1B. PSC sera yielded a predominantly homogenous (mushy) cytoplasmic staining pattern with DNase treated neutrophils (Fig. 1D).

EXAMPLE IX
IMMOBILIZATION OF Neutrophils ON MICROTITER PLATE

1. Count cells in suspension of step 7 of Example II using a microscope and hemacytometer and re-suspend cells in sufficient volume of 1X HBSS to achieve 2.5×10^6 cells per ml. Add 0.1 ml per well to a 96-well microtiter Immulon 1™ or Immulon™ plate (available from Dynatech Laboratories of Chantilly, Virginia) and let settle for 30-60 minutes.

2. Pull supernatant with 8 channel manifold connected to a vacuum and let plate air dry (approximately 2 hours) or turn upside down on the grate of a laminar flow hood to dry (approximately 10 minutes).

3. Fix cells to well by incubating cells for 10 minutes in 0.1 ml of 100% methanol per well. Discard methanol and let plate air dry. Store at -20°C.

EXAMPLE X

DNase TREATMENT OF NEUTROPHILS IMMOBILIZED
ON MICROTITER PLATE

A DNase solution is prepared by combining 3 units of
Promega RQ1™ DNase per ml buffer containing 40 mM of Tris-
HCl (pH 7.9), 10 mM sodium chloride, 6 mM magnesium chloride
and 10 mM calcium chloride.

Rinse plates prepared in accordance with Example VII
once with 25 ml phosphate buffered saline. Incubate
immobilized neutrophils in 0.1 ml of DNase solution per well
for about 30 minutes at 37°C. Wash the wells three times
with a total of about 100 ml phosphate buffered saline.
Block the wells by adding 0.15 ml of 0.25% bovine serum
albumin in phosphate buffered saline (pH 7.4) and allowing
to stand at room temperature for about one hour. Discard
blocking fluid.

EXAMPLE XI

DNase-TREATED, FIXED NEUTROPHIL ELISA

1. Add 0.1 ml human sera diluted as desired with
    phosphate buffered saline containing 0.25% bovine
    serum albumin to each well of the microtiter
    plates prepared in accordance with Example VIII
    and Example VII (i.e., with and without the DNase
    treatment). Add 0.01 ml phosphate buffered serum
    containing 0.25% bovine serum albumin to blank
    wells. Let stand at room temperature for one
    hour, in sufficient humidity to minimize volume
    loss.

2. Aspirate serum. Wash three times with a total of
    about 100 ml phosphate buffered saline containing
    0.02% sodium azide (NaN_3) and 0.05% Tween.

3.   Add to each well 0.1 ml of a 1:1000 dilution of alkaline phosphatase-coupled goat anti-human IgG antibody in phosphate buffered saline containing 0.25% bovine serum albumin. Goat F(ab')$_2$ anti-human IgG(Fc)-alkaline phosphatase may be obtained from Jackson Immuno-Research Laboratories in West Grove, Pennsylvania. Incubate for one hour at room temperature in sufficient humidity to minimize volume loss.

4.   Wash three times with a total of 100 ml phosphate buffered saline containing 0.02% sodium azide (NaN$_3$) and 0.05% Tween. Wash three more times with TRIS-NaCl solution containing 0.05 M Tris, 0.15 M NaCl, and 0.02% sodium azide, pH 7.5.

5.   Combine 0.75g disodium p-nitrophenol phosphate (United States Biochemicals catalogue #19587 or AMRESCO catalogue #P0364) with a Tris buffer containing 75 mM Tris-HCl, 1.5 mM MgCl$_2$, 0.02% sodium azide, pH 8.6 to form a substrate containing solution. Add 0.01 ml substrate containing solution to each well. Incubate at room temperature for 60 to 90 minutes in sufficient humidity to minimize volume loss, until blank wells reach 0.8 in absorbance.

6.   Read plate at 405 nm in an EMAX Microplate Reader (Molecular Devices, Menlo Park, California)

EXAMPLE XII

CHANGE IN ANCA BINDING TO DNase TREATED NEUTROPHILS RELATIVE TO CONTROL UNTREATED CELLS USING DNase-TREATED, FIXED NEUTROPHIL ELISA

In a panel of p-ANCA positive UC sera, the subset found to lose greater than 50% of ANCA binding by ELISA

corresponds to those that lost most or all of the p-ANCA
staining by immunofluorescent staining. On the other hand,
sera showing less than about 50% reduction in ANCA binding
by ELISA were found to display a p-ANCA pattern that

5   converted to cytoplasmic staining after DNase digestion of
neutrophils. In this latter group was also found a few sera
with a mixture of perinuclear/cytoplasmic staining pattern
that retained only the cytoplasmic pattern post DNase
treatment. The one serum displaying a cytoplasmic ANCA

10 staining pattern was found to have increased ANCA binding
post DNase treatment. The majority (4 out of 6) of p-ANCA
positive PSC sera lost less than 50% of the ANCA binding
after DNase treatment of neutrophils; in contrast only 5 out
of 14 UC p-ANCA positive sera showed such a loss. By

15 immunofluorescent staining these PSC sera were found to
display a p-ANCA staining pattern that became cytoplasmic
after DNase treatment.

Thus, the DNase-treated, fixed neutrophil ELISA may be
used to distinguish UC and PSC from CD, as well as other

20 types of inflammatory conditions of the intestines. The
unique perinuclear/cytoplasmic staining patterns associated
with immunofluorescent-type assays confirms the reliability
of ELISA assay and may allow further distinctions between UC
and PSC.

25   EXAMPLE XIII
              ANCA IN PEDIATRIC ULCERATIVE COLITIS

In the pediatric population, distinguishing between
UC, Crohn's disease (CD) and allergic colitis in children
with rectal bleeding (RB) is particularly difficult. Since

30 the occurrence of ANCA in adult patients with UC has been
well established, studies were undertaken to determine the
relationship between the occurrence of ANCA and pediatric

UC. To determine whether the presence of ANCA, as measured by DNase-treated fixed-neutrophil ELISA is sensitive and specific for pediatric UC, serum from children with UC (mean age=13), CD (mean age=14), RB (mean age=3) and other gastrointestinal inflammatory disorders (mean age=8) were tested in a blinded fashion. All ELISA positive samples were examined using immunofluorescence assay described above to determine ANCA staining patterns. ANCA was expressed as a percentage of UC positive sera binding and defined as positive when the value exceeded 2 standard deviations above the mean for normal control sera ($\geq$)12%. The results are presented in Table 3.

TABLE 3
ANCA IN PEDIATRIC ULCERATIVE COLITIS

	# Patients	#ANCA+ (%)	MEAN % Positive Cont.		MEAN Reciprocal Titer	
			Total	ANCA+	Total	ANCA+
UC	29	21 (72)	44	57	527	705
CD	41	7 (17)	8	16	61	114
RB	13	3 (23)	8	17	87	208
Non-IBD	94	7 (7)	6	21	63	229

UC=ulcerative colitis
CD=Crohn's disease
RB=rectal bleeding

Seventy-two percent of children with UC were ANCA positive compared to 17% with CD, 23% with RB and 7% with other gastrointestinal inflammatory disorders (Table 1). The mean percent of positive control at 1:100 dilution was also significantly higher in UC (p<0.00 vs CD and non-IBD, p<0.01 vs RB). In addition, mean titers of ANCA positive

samples were significantly higher, making ELISA titer very
specific for UC. The presence of a perinuclear
immunofluorescence pattern correlated with titer. It is
therefore seen that ANCA is sensitive (72%) and specific
5   (89%) for UC versus other inflammatory disorders.

EXAMPLE XIV
ANTIGEN REACTIVE WITH p-ANCA OF UC AND PSC
IS TRITON X-100™ INSOLUBLE

1. Count cells in suspension of step 7 of Example II
10   using a microscope and hemacytometer and re-
        suspend cells in sufficient volume of phosphate
        buffered saline containing 1.0% Triton X-100™ to
        acheive 2.5X10⁶ cells per ml phosphate buffered
        saline containing 0.5% Triton X-100™. Allow to
15   incubate on ice for about 10 minutes.

2. Cytocentrifuge onto glass slide as described in
    Example III, step 2.

3. Fix cytocentrifuged Triton X-100™ extract in
    accordance with the procedure set forth in Example
20   III, step 3.

4. Add 0.05 ml of a 1:20 dilution of UC p-ANCA
    positive serum or PSC p-ANCA positive serum in
    phosphate buffered saline to slides. Add 0.05 ml
    phosphate buffered saline to clean slides as
25   blanks. Incubate for 30 minutes to one hour at
        room temperature in sufficient humidity to
        minimize volume loss.

5. Process slides in accordance with the
    immunofluorescence assay of Example V, steps 2-5.

30   After Triton X-100™, neutrophil morphology was clearly
lost with no evidence of a clear nuclear structure upon

40

reaction with anti-DNA serum. However, cellular DNA was not
lost during Triton X-100™ treatment. Both UC p-ANCA
positive sera and PSC p-ANCA positive sera showed strong
reactivity with fixed Triton X-100™ neutrophil extract.
5   Based upon Triton X-100™ insolubility, an enriched fraction
of UC and PSC p-ANCA antigens can be prepared to isolate the
antigens.

EXAMPLE XV
LIBRARY CONSTRUCTION

10   V$_H$- and V$_L$-encoding DNA homolog libraries of the heavy
and light chain gene repertoire of lamina propria
lymphocytes (LPL) cells from humans diagnosed with UC and
seropositive for p-ANCA in a fixed neutrophil ELISA were
randomly combined, expressed and the resulting antibody
15   material screened for ability to bind neutrophil using a
phage display technique. The antibody material having
immunoreactivity with neutrophil were then screened for p-
ANCA staining pattern and for loss of the p-ANA staining
pattern using DNase-treated neutrophil as means of
20   identifying p-ANCA associated with UC.
        These variable heavy and light chain libraries were
constructed by PCR cloning of variable heavy and light
chains from these LPL. The homologs from these libraries
were randomly paired in the dicistronic phagemid expression
25   vector pComb 3 as described herein, resulting in a variable
heavy chain fusion protein containing the V$_H$ polypeptide and
a fragment of the filamentous phage coat protein III. E.
coli were subsequently transformed with these vectors
containing the DNA-encoding heterodimeric antibody material.
30   Expression of the vectors was induced and the cells
transformed with helper phage. Phage that were extruded
from the transformed E. coli encapsulated the vector DNA

encoding the nucleotide sequence and displayed the encoded
heavy and light chains as Fab antibody material anchored to
the phage coat via the gene III anchor protein. This
phagemid expression system thus links both the process of
recognition and replication in a single phage particle.

In a process called panning as described by Parmley et
al., Gene, 74: 305-318 (1988), the phage expressing
heterodimeric antibody material having anti-neutrophil
immunoreactivity are enriched and isolated. The
heterodimeric antibody material is then assayed for further
the presence of p-ANCA associated with UC by alcohol-fixed
indirect immunofluorescence ("the IIF assay") and for loss
of a positive p-ANCA staining pattern in the IIF assay using
DNase-treated alcohol-fixed neutrophil.

V_H and V_L Library Generation

Nucleotide sequences encoding immunoglobulin protein
CDRs are highly variable. However, there are several
regions of conserved sequences that flank the V domains of
the light and heavy chains that contain substantially
conserved nucleotide sequences, i.e., sequences that will
hybridize to the same primer sequence.

Polynucleotide synthesis ("amplification") primers that
hybridize to these conserved sequences and incorporate
restriction sites into the DNA homolog produced, restriction
sites that are suitable for operatively ligating the DNA
homolog to a vector, were constructed. More specifically,
the primers are designed so that the resulting DNA homologs
produced can be inserted into an expression vector in
reading frame with the upstream translatable DNA sequence at
the region of the vector containing the directional ligation
means. Amplification with the primers described herein is
performed on cDNA templates produced from total RNA isolated

from LPL of a human diagnosed with UC and seropositive for
p-ANCA.

V_H Primers

For amplification of the V_H domains, primers are
designed to introduce cohesive termini compatible with
directional ligation into the unique Xho I and Spe I sites
of the Hc2 expression cassette of the pComb 3 phagemid
expression vector. In all cases, the 5' primers listed in
SEQ ID NOs: 10 through 16 are chosen to be complimentary to
the first strand cDNA in the conserved N-terminus region
(anti-sense strand).

Additional V_H amplification primers, including the
unique 3' primer, are designed to be complimentary to a
portion of the first constant region domain of gamma 1 heavy
chain mRNA (SEQ ID NO: 9). These primers will produce DNA
homologs containing polynucleotides coding for amino acids
from the V_H domain and the first constant region domain of
immunoglobulin heavy chains of the IgG isotype. These DNA
homologs can therefore be used to produce Fab fragments
rather than F_V.

Additional unique 3' primers designed to be hybridized
to similar regions of another class of immunoglobulin heavy
chain such as IgM, IgE and IgA are contemplated. Other 3'
primers that hybridize to a specific region of a specific
class of CH_1 constant region and are adapted for
transferring the V_H domains amplified using this primer to
an expression vector capable of expressing those V_H domains
with a different class of heavy or light chain constant
regions are also contemplated.

Amplification is performed in seven separate reactions,
each containing one of the 5' primers shown in SEQ ID NOs:
10 through 16, and a 3' primer shown in SEQ ID NO: 9. The
5' primers incorporate a Xho I site and the 3' primers

incorporate a Spe I restriction site for the insertion of
the V_H-encoding DNA homolog into the Hc2 expression cassette
of the pComb 3 phagemid expression vector. See, Barbas,
C.F. et al., Proceedings of the National Academy of Science,
5   88: 7978-7982 (1991), incorporated herein by reference.

V_L Primers

For amplification of the V_L domains, amplification
primers are constructed that hybridize to the conserved
sequences of immunoglobulin light chains and that
10   incorporate restriction sites that allow cloning the V_L-
encoding DNA homologs into the Lc2 expression cassette of
the pComb 3 phagemid expression vector cut with Sac I and
Xba I. The 5' primers (SEQ ID NOs: 18 through 20) are
designed to be complimentary to the first strand cDNA in the
15   conserved N-terminus region. These primers also introduce a
Sac I restriction endonuclease site to allow the V_L-encoding
DNA homologs to be cloned into the pComb 3 phagemid Lc2
expression cassette. The 3' V_L amplification primer (SEQ ID
NO: 17) is designed to hybridize to the constant region of
20   kappa cDNA and to introduce the Xba I restriction
endonuclease site required to insert V_L-encoding DNA
homologs into the pComb 3 phagemid Lc2 expression cassette.
These primers allow DNA homologs to be produced that encode
immunoglobulin light chains of the kappa isotype. These
25   primers make it possible to produce a Fab fragment rather
than a Fv.

Amplification of the immunoglobulin light chain gene
repertoire is performed in three separate reactions, each
containing one of the 5' primers (SEQ ID NOs: 18 through 20)
30   and one of the 3' primers (SEQ ID NO: 17). The 5' primers
contain a Sac I restriction site and the 3' primers contain
the Xba I restriction site.

44

Amplification primers designed to amplify human light chain variable regions of the lambda isotype are also contemplated.

All primers and synthetic polynucleotides described
5   herein, were purchased from Oligos etc. (Wilsonville, OR). The pComb 3 expression vector was provided as a gift from Dr. Carlos Barbas III of the Scripps Research Institute, La Jolla, CA.

V_H and V_L Library Construction
10   Total RNA was extracted from 1.15×10^7 lymphocytes using standard guanadinium isothiocynate extraction protocols. See, for example, Chomcynski, P. and Saochi, N., Anal. Biochem. 162:156-159 (1987), incorporated herein by reference.

15   In preparation for PCR amplification, the RNA, prepared above, is used as a template for cDNA synthesis by a primer extension reaction. Thus, 10 μg RNA was reverse transcribed to single-stranded cDNA using 1 μg oligo-dT primer with 10 mM dithiothreitol, RNasin™ (a protein RNase inhibitor of
20   Promega Corporation, Madison, WI), 25 mM each dATP, dCTP, dGTP, dTTP, 1x reverse transcriptase buffer (Bethesda Research Laboratories, Bethesda, MD), and 2μl (two hundred units) reverse transcriptase (Superscript, Bethesda Research Laboratories) in 50 μl volume for 10 minutes at room
25   temperature followed by 50 minutes at 42°C. Following a 5 minute 90°C heat kill and 10 minutes on ice, the reaction was treated with 1 μl (one unit) RNase H (Bethesda Research Laboratories) for 20 minutes at 37°C.

The single-stranded cDNA generated above was amplified
30   using the polymerase chain reaction ("PCR") method. Family specific variable region and isotype specific constant region primers as described below were used to create heavy

chain IgG1 V$_H$1-V$_H$6 and kappa light chain V$_L$1-V$_L$3 specific
libraries:

Primer to create IgG1 heavy chain constant region library:
CG1z   5' GCATGTACTAGTTTTGTCACAAGATTTGGG 3' (SEQ ID NO:9)

5   Primers to create heavy chain variable region library:
V$_H$1a   5' CAGGTGCAGCTCGAGCAGTCTGGG   3'   (SEQ ID NO:10)
V$_H$2f   5' CAGGTGCAGCTACTCGAGTCGGG   3'   (SEQ ID NO:11)
V$_H$3a   5' GAGGTGCAGCTCGAGGAGTCTGGG   3'   (SEQ ID NO:12)
V$_H$3f   5' GAGGTGCAGCTGCTCGAGTCTGGG   3'   (SEQ ID NO:13)
10 V$_H$4f   5' CAGGTGCAGCTGCTCGAGTCGGG   3'   (SEQ ID NO:14)
V$_H$6a   5' CAGGTACAGCTCGAGCAGTCAGG   3'   (SEQ ID NO:15)
V$_H$6f   5' CAGGTACAGCTGCTCGAGTCAGGTCCA 3' (SEQ ID NO:16)

Primer to create Kappa light chain constant region library:
C$_K$1d   5' GCGCCGTCTAGAACTAACACTCTCCCCTGTTGAAGC
15   TCTTTGTGACGGGCGATCTCAG 3'   (SEQ ID NO:17)

Primer to create Kappa light chain variable region library:
V$_K$1a   5' GACATCGAGCTCACCCAGTCTCCA 3' (SEQ ID NO:18)
V$_K$2a   5' GATATTGAGCTCACTCAGTCTCCA 3' (SEQ ID NO:19)
V$_K$3a   5' GAAATTGAGCTCACGCAGTCTCCA 3' (SEQ ID NO:20)

20   PCR amplification is performed in a 100μl reaction
containing the products of the reverse transcription
reaction (about 1 μl of 450 μl reaction of the single-
stranded cDNA), 60 pm of 3'V$_H$ primer (SEQ ID NO: 9), 60 pm
of the 5' primer (one of SEQ ID NOs: 10 through 16), 8 μl of
25 the mixture of dNTP's at 25 mM each, 10 μl of 10 x PCR
Buffer (Perkin-Elmer), and 5 units of Tag DNA polymerase
(Perkin-Elmer, Norwalk, CT). The reaction mixture is
subjected to 30 cycles of amplification using a Perkin-Elmer
9600 thermocycler. Each amplification cycle included

denaturing of cDNA at 94°C for 15 seconds, followed by annealing of primers at 52°C for 50 seconds, and amplification at 72°C for 90 seconds. This was followed by a 10 minute extension at 72°C. Efficient and reproducible

5   DNA homolog synthesis was achieved with the primers defined herein, producing amplified cDNA V_H-encoding homologs having a major band of about 680 bp and amplified cDNA V_K-coding homologs having a major band at about 660 bp.

After verifying by agarose gel electrophoresis that all

10 amplifications were successful and that similar yields were achieved, the V_H-encoding and V_L-encoding DNA homologs were separately pooled and gel purified on 0.8% Seaplaque GTG Agarose (FMC, Rockland, ME) according to the manufacturer's directions.

15   Ligation of V_L-encoding DNA Homologs into Vector

Equal portions of the products from each light chain primer extension reaction were mixed to generate a pooled V_L library of UC. The pooled V_L library was double-digested with 70 units XbaI per microgram pooled V_L library and 35

20 units SacI per microgram pooled V_L library. (All restriction enzymes are available from Boehringer-Mannheim, Indianapolis, IN.) Digested products were again gel purified as described above, and the region of the gel containing DNA fragments of about 660 bp was excised,

25 extracted from agarose and ethanol precipitated. The resulting V_L DNA homologs represent a repertoire of kappa light chain polypeptide genes having cohesive termini adapted for directional ligation to the pComb 3 phagemid Lc2 expression cassette.

30   The pComb 3 phagemid Lc2 expression cassette is prepared for inserting a light chain DNA homolog by admixing 30 μg of the phagemid to a solution containing 280 units of Xba I and 160 units of Sac I restriction endonucleases and a

buffer recommended by the manufacturer. This solution was
maintained at 37°C for 3 hours. The solution was
precipitated with 2 ml glycogen, 1/10 volume 3M NaAc, 2.5
volume ethanol, at -20°C for 1 hour, then pelleted and

5   washed with 70% ethanol. The pellet was re-suspended in
water and gel purified on .8% 1 x TAE Seplaque 676. A 4 Kb
band was excised, phenol extracted, LiCl₃ treated and
ethanol precipitated the same as PCR products.

The Lc2 expression cassette was then ready for ligation

10 with the V$_L$-encoding DNA homologs prepared above. These V$_L$-
encoding DNA homologs were then directly inserted into the
Xba I and Sac I restriction digested Lc2 expression cassette
by ligating 0.45 μg of V$_L$ DNA homolog into 1.4 μg of
digested pComb 3 (kindly provided by Dr. Carlos Barbas III

15 of the Scripps Research Institute, La Jolla, California and
described in Barbas et al., Proc. Natl. Acad. Sci. USA
88:7978-7982 (1991), incorporated herein by reference) using
10 units ligase in 200 μl volume ligase buffer stored
overnight at 25°C, and then heat killed by maintaining at

20 65°C for 15 minutes (Boehringer-Mannheim). DNA was
precipitated, washed with 70% ethanol, and re-suspended in
15 μl 10 mM MgCl₂.

Transformation of Host with Vector Containing V$_L$
Library

25   Escherichia coli XLI-Blue cells (Stratagene, La Jolla,
CA) were transformed with re-suspended DNA by
electroporation: 300 μl of stock made by concentrating 1
liter of E. coli OD$_{600}$ = .8 down to 4 ml of cells were
electroporated with 15 μl DNA (= 2 μg) (all of ligation

30 mix). Transformed cells were selected for by plasmid
antibiotic resistance by growth super broth containing 100
μg/ml carbenicillin. The library size was 8.6 x 10^7
transformants with 6% background re-ligation.

Antibiotic resistant colonies were amplified by growth
in liquid cultures at 37°C in super broth ("SB") medium
(30 g tryptone, 20 g yeast extract, and 10 g 3[N-
Morpoholino] propane-sulfonic acid (Mops) per liter of
5   water, adjusted to pH 7) supplemented with 10 μg/ml
tetracycline, 20 μg/ml carbenicillin, 40 mM glucose and
10 mM MgCl₂. pComb 3 phagemids encoding a kappa V$_L$
polypeptide ("Kappa - pComb 3 phagemid") were isolated using
Qiagen-tips™, an anion-exchange resin of Qiagen, Chatsworth,
10   CA following manufacturer instructions. Isolated Kappa-
pComb 3 phagemids were double-digested with 10 units XhoI
and 3 units SpeI per microgram Kappa-pComb 3 phagemid.
Reaction mix was ethanol precipitated and 4.7 Kb double cut
phagemid was gel purified on .8% Seaplaque TAE gel as
15   before. The Kappa-pComb 3 phagemids were now ready for
ligation with the heavy chain library.

Ligation of V$_H$-encoding DNA Homologs into Vector and Transformation of Host

Equal portions of the products form each heavy chain
20   primer extension were mixed to generate a pooled V$_H$-encoding
DNA homolog library. The pooled V$_H$ library was prepared for
ligation into the Hc2 expression cassette of the Kappa-
pComb 3 phagemid by digestion with Xho I and Spe I
nucleases. Accordingly, the pooled V$_H$ library was double-
25   digested with 70 units XhoI and 17 units SpeI per microgram
pooled V$_H$ library. Then, .40 μg digested heavy chain
library was ligated with 1.4 μg digested Kappa-pComb 3
phagemid, described above, using 10 units ligase in 200 μl
volume ligase buffer. The reaction was stopped by a heat
30   kill at 65°C for 15 minutes. DNA was precipitated, the
pellet re-suspended in 15 μl 10 mM MgCl₂ and used to
electroporate E. coli XLI-Blue cells. Electroporated cells
were grown in SB, supplemented as described above, except

that glucose was not included. The library size was 4.9×10^7 with 14% background re-ligation after heavy chain cloning. Presence of both V_H-and V_L-encoding DNA homologs in the vector was verified by restriction analysis, seven out of seven clones contains both homologs.

Ten milliliter cultures of electroporated E. coli XLI-Blue cells were then transferred to SB supplemented with 50 μg/ml carbenicillin, 10 μg/ml tetracycline, and 10 mM $MgCl_2$ and incubated for another hour. Cultured cells were then infected with 10^{12} VCS-M13 helper phage (Stratagene, La Jolla, CA) to initiate the generation of copies of the sense strand of the phagemid DNA. After adding helper phage the mixture was added to 100 ml of SB supplemented with 50 μl/ml carbenicillin, 10 μl/ml tetracycline, and 10 mM $MgCl_2$. The admixture containing the helper phage was then maintained for an additional 2 hours at 37°C to allow for filamentous bacteriophage assembly wherein the expressed heterodimeric antibody material of UC+ fused to cpIII bacteriophage anchor domain were incorporated into the surface of the bacteriophage particle. After 2 hours the mixture was spiked with 70 μg/ml kanamycin to select for helper phage infected E. coli and then allowed to grow overnight at 37°C, 300 rpm. The phage were precipitated by centrifugation resulting in a bacterial cell pellet and a supernatant containing phage, with the titer of colony-forming units ("CFU") determined by plating on LB plates with 100 μg/ml carbenicillin.

EXAMPLE XVI

PANNING

Each well of a 24-well microtiter plate was coated with methanol-fixed neutrophils by adding 10^8 neutrophils, allowing them to settle, air dry and then fixing with 100%

methanol. Each well was blocked for one hour at 37°C with
3% bovine serum albumin ("BSA") in Tris-buffered saline
("TBS"). Blocking solution was removed and 5 x 10^{11} phage in
250 μl TBS was added and allowed to incubate for two hours

5   at 37°C. After washing, acid elution, and neutralization,
the number of phage eluted was monitored by CFU.

Eluted phage were amplified by reinfecting E. coli XLI-
Blue and the panning/amplification cycle repeated five
times until there was at least 100 fold enrichment. In this

10   manner a library of phage enriched for p-ANCA material
having immunoreactivity with neutrophil antigen was
generated. For enrichment quantitation, aliquots of the
original library were re-panned in parallel with each cycle
of enrichment to control for daily fluctuations in phage

15   recovery. Enrichment was calculated by ratio of phage on
vs. off and compared to the unenriched library run on the
same day. Panning was also performed in a 96 well format
with 10^{11} phage per well to compare formats.

EXAMPLE XVII
20   ### PREPARATION OF SOLUBLE RECOMBINANT ANTI-NEUTROPHIL
ANTIBODY MATERIAL OF UC AND LIBRARY SCREENING

Preparation of soluble heterodimeric antibody material,
specifically Fab, was performed by isolating phagemid using
Qiagen-tips™ in accordance with the manufacturer's

25   instructions. (Qiagen, Chatsworth, CA) Isolated phagemid
was then digested with 17 units SpeI and 50 units NheI per
microgram of phagemid to remove cpIII gene segment. The
phagemid DNA was then gel-purified and self-ligated by using
10 units ligase per 1 μg phagemid and maintaining the

30   reaction mixture overnight at 25°C. The reaction was stopped
by maintaining it at 65°C for 15 minutes. 200 ng gel
purified fragment was self ligated in 20 μl volume and used

to transform *E. coli* XLI-Blue by electroporation at 0°C in
.2 cm gap curette at 2.5 kV, 25 μF and 200 R using 40 μl of
E. coli stock and 1 μl of ligation mix. Single colonies
were picked from an LB agar plates containing 100 μl/ml
5   carbenicillin and grown in 10 ml SB supplemented with 10
μg/ml tetracycline, 50 μg/ml carbenicillin, and 20 mM MgCl₂
for six hours. Cultures were then induced by the addition
of 1 mM isopropyl 6-D-thiogalactopyranoside ("IPTG") (United
States Biochemicals, Cleveland, OH) and grown overnight.
10 The phage were isolated by centrifugation resulting in a
bacterial cell pellet and a supernatant containing phage.
The supernatant was removed and analyzed for Fab production
by kappa-capture ELISA as described above, detecting with
goat anti-human Fab-alkaline phosphatase (Pierce, Rockland,
15 IL). Ten clones each from the enriched and unenriched
libraries were selected for comparison. Six out of the ten
clones from the unenriched library produced significant
amounts of Fab as assayed by kappa-capture ELISA. In
contrast, ten out of ten clones from the enriched library
20 produced Fab, indicating that the enriched library had
positively selected for Fab expression.

    These clones were also analyzed for neutrophil binding
by alcohol-fixed neutrophil ELISA. None of the ten clones
from the unenriched library bound neutrophil, whereas all
25 samples clones from the enriched library demonstrated avid
neutrophil binding.

    Diversity of heavy and light chain usage in Fabs from
enriched and unenriched libraries were monitored by
digesting 4 μg of phagemid encoding a single Fab with 20
30 units of BSTN1 (New England Biolabs, Beverly, MA) and
analyzing fragments on a 3% agarose gel. Each of thirty
clones from the unenriched library showed a distinct
restriction pattern, whereas the clones from the enriched
library displayed only two clonal patterns. Clones

representative of these two patterns (5-3 and 5-4) were
therefore directly analyzed by DNA sequencing, as described
below.

EXAMPLE XVIII

PURIFICATION OF FAB

Both enriched and unenriched libraries were transferred
from pComb 3 to C₃AP313H₆, a pComb 3 derivative which fuses
six histidine to the carboxy terminus of the Fab after SpeI
and NheI digestion to remove the cpIII anchor domain.
(C₃AP313H₆ was a gift of Carlos Barbas III, Scripps Research
Institute, La Jolla, California). Libraries were moved by
removing the V$_H$- and V$_L$- encoding polynucleotides from the
Hc2 and Lc2 expression cassettes of pComb 3 and sequentially
ligating them into C₃AP313H₆. *E. coli* XLI-Blue cells were
transformed with the new phagemid by electroporation.
Individual colonies were isolated by LB agar selection
supplemented with 100 µl/ml carbenicillin.

The 5-3 clone from the enriched library was chosen for
large scale purification. A single colony was picked and
allowed to grow overnight in 10 ml SB, supplemented with 10
µg/ml tetracycline, 50 µg/ml carbenicillin, 10 mM MgCl₂, and
40 mM glucose. The bacterial culture was pelleted by
centrifugation to remove glucose and the cell pellet
transferred into one liter of SB containing 50 µg/ml
carbenicillin and 20 mM MgCl₂. The XL1-Blue cells were
grown at 37°C shaking at 300 rpm until absorbance (OD₆₀₀) was
between 0.6-0.8. The cell culture was then induced with 4
mM IPTG to express the heterodimeric antibody material and
grown at 30°C overnight. The cell culture was centrifuged
to pellet the XL1-Blue cells and the pellet re-suspended in
30 ml sonication buffer (50 mM NaPO₄, 300 mM NaCl, 0.01%
NaN₃, pH 7.9). The re-suspended cells were sonicated eight

times in 15 seconds bursts at 50% power (40 watts micro
sonic disrupter, Tekmar, Cincinnati, OH).

The sonicate was centrifuged at 15,000 rpm in a Beckman
JA-20 centrifuge for 40 min at 4°C and the supernatant
serially filtered through a 0.45 and a 0.22 micron Nytex
filter (Amicon, Beverly, MA). Sonicate was immediately
loaded at 20 ml/hr on a 1 ml NTA-Ni column (Qiagen) and
washed with sonication buffer, typically 40-50 ml, until
absorbance (OD_{280}) was <0.01. The column was then washed
with 10 ml of 10 mM imidazole in sonication buffer to remove
contaminants, followed with 10 ml each of 100 mM, 250 mM,
and 500 mM imidazole collecting 1 ml fractions monitored by
OD_{280}. Aliquots were analyzed by SDS-PAGE 12% denaturing and
reducing gel to determine where Fab eluted. Due to the
presence of imidazole, samples with loading dye were not
boiled, but denatured instead at 37°C for 10 min before
loading. Typically, the Fab elutes in first 3 fractions of
the 100 mM imidazole wash.

One milliliter fractions that contain Fab were then
pooled and dialyzed (6-8 kD cutoff membranes) using Amicon
dialysis membranes against PBS to remove imidazole. Samples
were concentrated and any free heavy or light chain removed
using a Centricon 50™, centrifugation-dialysis membrane from
Amicon Corporation, Beverly, MA.

Curiously, the calculated antibody level in the
purified fraction differed with total protein (Bio-rad
Protein Assay, Richmond, CA) versus ELISA (anti-Kappa)
determination. Per 1 liter bacterial culture, Fab yield was
~1 mg by total protein assay, versus ~0.1 mg by immunoassay.
Since use of the proteins in this study utilized ELISA
immunoreactivity, Fab concentrations are reported using the
ELISA method.

The 5-3 Fab was characterized using the assays
described herein. Strong binding (approx. 0.1

micrograms/milliliter) to fixed neutrophil in an ELISA
format. It is also notable that 5-3 p-ANCA Fab is avid
compared to UC serum, since optimal binding occurred at 1%
serum (or approx. 0.1 milligrams/milliliter total IgG).

5   Estimating that approx. 1% hyperimmune serum is antigen-
specific, then the level of native p-ANCA IgG is approx. 1
microgram/ml, or similar in range to binding by monovalent
Fab.

In inflammatory disorders, ANCA-type marker antibodies

10   are specific for certain defined neutrophil proteins. The
5-3 p-ANCA Fab was tested for immunoreactivity with
cathepsin G, elastase, myeloperoxidase and lactoferrin in an
ELISA format. No binding was detected up to 500
nanograms/ml of 5-3 p-ANCA Fab.

15   The 5-3 p-ANCA Fab was also tested by alcohol-fixed
neutrophil IIF assay for the p-ANCA staining pattern.
Immunofluorescent detection of neutrophil staining by 5-3 p-
ANCA Fab yielded the same p-ANCA staining pattern produced
by conventional UC serum. When the immunoreactivity of 5-3

20   p-ANCA Fab was tested for DNase sensitivity in accordance
with EXAMPLE VI above, as with conventional p-ANCA
seropositive UC serum, DNase I treatment of neutrophil
caused the complete loss of detectable p-ANCA staining
pattern. In addition, confocal microscopy demonstrated that

25   5-3 p-ANCA Fab binds antigen located inside the nuclear
envelop, a characteristic found in p-ANCA seropositive UC
serum.

EXAMPLE XIX

NUCLEIC ACID SEQUENCING

30   Nucleic acid sequencing was carried out on double-
stranded DNA of the 5-3 and 5-4 clones using 5' and 3'
primers for the heavy and light chains (SEQ ID NOs: 21 and

22, and SEQ ID NOs. 23 and 24, respectively) and Sequenase
1.0 (United States Biochemicals). Homology searches and
lineups were performed using Genebank.

5   Although the invention has been described with
reference to presently preferred embodiments, it should be
understood that various modifications can be made without
departing from the spirit of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT:
        (A) NAME: Cedars-Sinai Medical Center
        (B) STREET: 8700 Beverly Boulevard
        (C) CITY: Los Angeles
        (D) STATE: California
        (E) COUNTRY: US
        (F) POSTAL CODE (ZIP): 90048-1863
        (G) TELEPHONE: (310) 855-5284
        (H) TELEFAX: (310) 967-0101

    (ii) TITLE OF INVENTION: Methods for Selectively Detecting Perinuclear Anti-Neutrophil Cytoplasmic Antibody of Ulcerative Colitis, Primary Sclerosing Cholangitis, or Type I Autoimmune Hepatitis

    (iii) NUMBER OF SEQUENCES: 24

    (iv) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

    (vi) PRIOR APPLICATION DATA:
        (A) APPLICATION NUMBER: US 08/196,003
        (B) FILING DATE: 11-FEB-1994

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 699 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: circular

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (v) FRAGMENT TYPE: N-terminal

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapiens
        (F) TISSUE TYPE: Gut-associated lymphoid
        (G) CELL TYPE: Lymphocyte

    (vii) IMMEDIATE SOURCE:

        (B) CLONE: 5-3

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 1..699
        (D) OTHER INFORMATION: /codon_start= 1
            /product= "Human Heavy Chain of IgG ANCA
            associated with UC"

    (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 1..15
        (D) OTHER INFORMATION: /product= "N-Terminal Tag"

    (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 16..96
        (D) OTHER INFORMATION: /label= FR1
            /note= ""FR1" refers to Framework Region 1"

    (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 97..111
        (D) OTHER INFORMATION: /label= CDR1
            /note= ""CDR1" refers to Complimentarity
            Determining Region 1"

    (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 112..153
        (D) OTHER INFORMATION: /label= FR2
            /note= ""FR2" refers to Framework Region 2"

    (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 154..204
        (D) OTHER INFORMATION: /label= CDR2
            /note= ""CDR2" refers to Complimentarity
            Determining Region 2"

    (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 205..300
        (D) OTHER INFORMATION: /label= FR3
            /note= ""FR3" refers to Framework Region 3"

    (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 301..327
        (D) OTHER INFORMATION: /label= CDR3
            /note= ""CDR3" refers to Complimentarity
            Determining Region 3"

    (ix) FEATURE:
        (A) NAME/KEY: misc_RNA

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        (B) LOCATION: 328..360
        (D) OTHER INFORMATION: /label= FR4
              /note= ""FR4" refers to Framework Region 4"

   (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 361..651
        (D) OTHER INFORMATION: /label= CH1
              /note= ""CH1" refers to Constant Segment 1 of the
              Heavy Chain"

   (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 652..678
        (D) OTHER INFORMATION: /label= Hinge
              /note= ""Hinge" refers to Partial Hinge Segment of
              the Heavy Chain"

   (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 679..699
        (D) OTHER INFORMATION: /label= Hex-HTAG
              /note= ""Hex-HTAG" refers to Hexahistidine Tag"

   (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 16..651
        (D) OTHER INFORMATION: /label= Fd
              /note= ""Fd" refers to the Fd of the Heavy Chain"

   (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 16..300
        (D) OTHER INFORMATION: /label= VHSEGMENT
              /note= ""VHSEGMENT" refers to Variable Segment of
              the Heavy Chain"

   (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 301..315
        (D) OTHER INFORMATION: /label= D
              /note= ""D" refers to Diversity Segment"

   (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 316..360
        (D) OTHER INFORMATION: /label= JH
              /note= ""JH" refers to Joining Segment of the
              Heavy Chain"

   (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 16..360
        (D) OTHER INFORMATION: /label= VHDOMAIN
              /note= ""VHDOMAIN" refers to Variable Domain of
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the Heavy Chain"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GCC CAG GTG AAA CTG CTC GAG CAG TCT GGG GGA GGC GTG GTC CAG CCT
48
Ala Gln Val Lys Leu Leu Glu Gln Ser Gly Gly Gly Val Val Gln Pro
1               5                   10                  15

GGG AAG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGG
96
Gly Lys Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg
            20                  25                  30

AAC TAT GGC ATG CAC TGG GTC CGG CAG GCT CCA GGC AAG GGG CTG GAG
144
Asn Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
            35                  40                  45

TGG GTG GCA GGT ATT TCC TCT GAT GGA AGA AAA AAG TAT GTA GAC
192
Trp Val Ala Gly Ile Ser Ser Asp Gly Arg Lys Lys Tyr Val Asp
        50                  55                  60

TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAG TCC AAG AAC ACG
240
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Lys Ser Lys Asn Thr
 65                  70                  75                  80

CTG TAT CTG CAA ATG AAC AGC CTC AGA GCT GAG GAC ACG GCT GTG TAT
288
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95

TAC TGT GCG AAA TTG TCC CGC GCG GGT GGT TTT GAC ATC TGG GGC CAA
336
Tyr Cys Ala Lys Leu Ser Arg Ala Gly Gly Phe Asp Ile Trp Gly Gln
            100                 105                 110

GGG ACA ATG GTC ACC GTC TCT TCA GCC TCC ACC AAG GGC CCA TCG GTC
384
Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
            115                 120                 125

TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC
432
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
            130                 135                 140

CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG
480
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160
```

TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC
528
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
              165   170   175

CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC
576
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
              180   185   190

TCC AGC AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG
624
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
              195   200   205

CCC AGC AAC ACC AAG GTG GAC AAG AAA GCA GAG CCC AAA TCT TGT GAC
672
Pro Ser Asn Thr Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp
      210   215   220

AAA ACT AGT CAC CAC CAC CAC CAC CAC   699
Lys Thr Ser His His His His His His
225   230

(2) INFORMATION FOR SEQ ID NO:2:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 233 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ala Gln Val Lys Leu Leu Glu Gln Ser Gly Gly Val Val Gln Pro
  1   5   10   15

Gly Lys Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg
              20   25   30

Asn Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
          35   40   45

Trp Val Ala Gly Ile Ser Ser Asp Gly Arg Lys Lys Tyr Val Asp
      50   55   60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Lys Ser Lys Asn Thr
65   70   75   80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                  85   90   95

Tyr Cys Ala Lys Leu Ser Arg Ala Gly Gly Phe Asp Ile Trp Gly Gln

                100   105   110

Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
            115   120   125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
        130   135   140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145   150   155   160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165   170   175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180   185   190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
            195   200   205

Pro Ser Asn Thr Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp
    210   215   220

Lys Thr Ser His His His His His His
225   230

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 732 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: circular

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (v) FRAGMENT TYPE: N-terminal

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapiens
        (F) TISSUE TYPE: Gut-associated lymphoid
        (G) CELL TYPE: Lymphocyte

    (vii) IMMEDIATE SOURCE:
        (B) CLONE: S-4

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 1..732
        (D) OTHER INFORMATION: /codon_start= 1
            /product= "Human Heavy Chain of IgG ANCA

associated with UC"

(ix) FEATURE:
     (A) NAME/KEY: misc_RNA
     (B) LOCATION: 1..15
     (D) OTHER INFORMATION: /product= "N-Terminal Tag"

(ix) FEATURE:
     (A) NAME/KEY: misc_RNA
     (B) LOCATION: 16..93
     (D) OTHER INFORMATION: /label= FR1
          /note= ""FR1" refers to Framework Region 1"

(ix) FEATURE:
     (A) NAME/KEY: misc_RNA
     (B) LOCATION: 94..108
     (D) OTHER INFORMATION: /label= CDR1
          /note= ""CDR1" refers to Complimentarity
          Determining Region 1"

(ix) FEATURE:
     (A) NAME/KEY: misc_RNA
     (B) LOCATION: 109..150
     (D) OTHER INFORMATION: /label= FR2
          /note= ""FR2" refers to Framework Region 2"

(ix) FEATURE:
     (A) NAME/KEY: misc_RNA
     (B) LOCATION: 151..201
     (D) OTHER INFORMATION: /label= CDR2
          /note= ""CDR2" refers to Complimentarity
          Determining Region 2"

(ix) FEATURE:
     (A) NAME/KEY: misc_RNA
     (B) LOCATION: 202..297
     (D) OTHER INFORMATION: /label= FR3
          /note= ""FR3" refers to Framework Region 3"

(ix) FEATURE:
     (A) NAME/KEY: misc_RNA
     (B) LOCATION: 298..360
     (D) OTHER INFORMATION: /label= CDR3
          /note= ""CDR3" refers to Complimentarity
          Determining Region 3"

(ix) FEATURE:
     (A) NAME/KEY: misc_RNA
     (B) LOCATION: 361..393
     (D) OTHER INFORMATION: /label= FR4
          /note= ""FR4" refers to Framework Region 4"

(ix) FEATURE:
     (A) NAME/KEY: misc_RNA
     (B) LOCATION: 394..684

                    (D) OTHER INFORMATION: /label= CH1
                         /note= ""CH1" refers to Constant Segment of the
                         Heavy Chain"

             (ix) FEATURE:
                    (A) NAME/KEY: misc_RNA
                    (B) LOCATION: 685..711
                    (D) OTHER INFORMATION: /label= Hinge
                         /note= ""Hinge" refers to Partial Hinge Segment of
                         the Heavy Chain"

             (ix) FEATURE:
                    (A) NAME/KEY: misc_RNA
                    (B) LOCATION: 712..732
                    (D) OTHER INFORMATION: /label= Hex-HTag
                         /note= ""Hex-HTag" refers to Hexahistidine Tag"

             (ix) FEATURE:
                    (A) NAME/KEY: misc_RNA
                    (B) LOCATION: 16..684
                    (D) OTHER INFORMATION: /label= Fd
                         /note= ""Fd" refers to the Fd of the Heavy Chain"

             (ix) FEATURE:
                    (A) NAME/KEY: misc_RNA
                    (B) LOCATION: 16..297
                    (D) OTHER INFORMATION: /label= VHSEGMENT
                         /note= ""VHSEGMENT" refers to Variable Segment of
                         the Heavy Chain"

             (ix) FEATURE:
                    (A) NAME/KEY: misc_RNA
                    (B) LOCATION: 298..363
                    (D) OTHER INFORMATION: /label= D
                         /note= ""D" refers to Diversity Segment"

             (ix) FEATURE:
                    (A) NAME/KEY: misc_RNA
                    (B) LOCATION: 364..408
                    (D) OTHER INFORMATION: /label= JH
                         /note= ""JH" refers to Joining Segment of the
                         Heavy Chain"

             (ix) FEATURE:
                    (A) NAME/KEY: misc_RNA
                    (B) LOCATION: 16..408
                    (D) OTHER INFORMATION: /label= VHDOMAIN
                         /note= ""VHDOMAIN" refers to Variable Domain of
                         the Heavy Chain"

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTC GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AAG TCC CTG AGA CTC
48

```
Leu Glu Ser Gly Gly Gly Val Val Gln Pro Gly Lys Ser Leu Arg Leu
 1               5                   10                  15

TCC TGT GCA GCC TCT GGA TTC ACC TTC AGG AAC TAT GGC ATG CAC TGG
96
Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asn Tyr Gly Met His Trp
                20                  25                  30

GTC CGG CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GGT ATT TCC
144
Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Gly Ile Ser
            35                  40                  45

TCT GAT GGA AGA AAA AAA AAG TAT GTA GAC TCC GTG AAG GGC CGA TTC
192
Ser Asp Gly Arg Lys Lys Lys Tyr Val Asp Ser Val Lys Gly Arg Phe
        50                  55                  60

TTC ATC TCC AGA GAC AAT TCC AAG AAC ACC CTG TAT CTG CAA TTG AAC
240
Phe Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Leu Asn
 65                  70                  75                  80

AGC CTG AGA GCT GAG GAC ACG GCT GTC TAT TAC TGT GCG AAA GAT GAG
288
Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Asp Glu
                85                  90                  95

TTT AGT TCT ACC CGG AAG AAC TTC TTG ACT GGT CAA TCA AAG ACC TTT
336
Phe Ser Ser Thr Arg Lys Asn Phe Leu Thr Gly Gln Ser Lys Thr Phe
                100                 105                 110

GCG GCC TAC TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG GTC ACC
384
Ala Ala Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr
            115                 120                 125

GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC
432
Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
        130                 135                 140

TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC
480
Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
145                 150                 155                 160

AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC
528
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
                165                 170                 175

CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
576
```

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
            180   185   190

CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC
·624
Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
            195   200   205

ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG
672
Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
            210   215   220

GTG GAC AAG AAA GCA GAG CCC AAA TCT TGT GAC AAA ACT AGT CAC CAC
720
Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys Thr Ser His His
225   230   235   240

CAC CAC CAC CAC
His His His His   732

(2) INFORMATION FOR SEQ ID NO:4:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 244 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Leu Glu Ser Gly Gly Gly Val Val Gln Pro Gly Lys Ser Leu Arg Leu
    1   5   10   15

Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asn Tyr Gly Met His Trp
            20   25   30

Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Gly Ile Ser
            35   40   45

Ser Asp Gly Arg Lys Lys Lys Tyr Val Asp Ser Val Lys Gly Arg Phe
        50   55   60

Phe Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Leu Asn
65   70   75   80

Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Asp Glu
                85   90   95

Phe Ser Ser Thr Arg Lys Asn Phe Leu Thr Gly Gln Ser Lys Thr Phe
            100   105   110

Ala Ala Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr
        115   120   125

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
        130   135   140

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
145   150   155   160

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
                165   170   175

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
                180   185   190

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
        195   200   205

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
        210   215   220

Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys Thr Ser His His
225   230   235   240

His His His His

(2) INFORMATION FOR SEQ ID NO:5:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 642 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: circular

        (ii) MOLECULE TYPE: cDNA

        (iii) HYPOTHETICAL: NO

        (iv) ANTI-SENSE: NO

        (vi) ORIGINAL SOURCE:
            (A) ORGANISM: Homo sapiens
            (F) TISSUE TYPE: Gut-associated lymphoid
            (G) CELL TYPE: Lymphocyte

        (vii) IMMEDIATE SOURCE:
            (B) CLONE: 5-3

        (ix) FEATURE:
            (A) NAME/KEY: CDS
            (B) LOCATION: 1..642
            (D) OTHER INFORMATION: /codon_start= 1
                /product= "Kappa Light Chain of ANCA associated
                with Ulcerative Colitis"

67

(ix) FEATURE:
    (A) NAME/KEY: misc_RNA
    (B) LOCATION: 1..3
    (D) OTHER INFORMATION: /label= N-TerminalTag

(ix) FEATURE:
    (A) NAME/KEY: misc_RNA
    (B) LOCATION: 4..285
    (D) OTHER INFORMATION: /label= VKSEGMENT
        /note= ""VKSEGMENT" refers to Variable Segment of
        the Kappa Light Chain"

(ix) FEATURE:
    (A) NAME/KEY: misc_RNA
    (B) LOCATION: 286..324
    (D) OTHER INFORMATION: /label= JK
        /note= ""JK" refers to Joining Segment of the
        Kappa Light Chain"

(ix) FEATURE:
    (A) NAME/KEY: misc_RNA
    (B) LOCATION: 325..642
    (D) OTHER INFORMATION: /label= CK
        /note= ""CK" refers to Constant Segment of the
        Kappa Light Chain"

(ix) FEATURE:
    (A) NAME/KEY: misc_RNA
    (B) LOCATION: 4..66
    (D) OTHER INFORMATION: /label= FR1
        /note= ""FR1" refers to Framework Region 1"

(ix) FEATURE:
    (A) NAME/KEY: misc_RNA
    (B) LOCATION: 67..102
    (D) OTHER INFORMATION: /label= CDR1
        /note= ""CDR1" refers to Complimentarity
        Determining Region 1"

(ix) FEATURE:
    (A) NAME/KEY: misc_RNA
    (B) LOCATION: 103..147
    (D) OTHER INFORMATION: /label= FR2
        /note= ""FR2" refers to Framework Region 2"

(ix) FEATURE:
    (A) NAME/KEY: misc_RNA
    (B) LOCATION: 148..168
    (D) OTHER INFORMATION: /label= CDR2
        /note= ""CDR2" refers to Complimentarity
        Determining Region 2"

(ix) FEATURE:
    (A) NAME/KEY: misc_RNA
    (B) LOCATION: 169..264

          (D) OTHER INFORMATION: /label= FR3
                /note= ""FR3" refers to Framework Region 3"

     (ix) FEATURE:
          (A) NAME/KEY: misc_RNA
          (B) LOCATION: 265..291
          (D) OTHER INFORMATION: /label= CDR3
                /note= ""CDR3" refers to Complimentarity
                Determining Region 3"

     (ix) FEATURE:
          (A) NAME/KEY: misc_RNA
          (B) LOCATION: 292..324
          (D) OTHER INFORMATION: /label= FR4
                /note= ""FR4" refers to Framework Region 4"

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCC GAG CTC ACG CAG TCT CCA GGC ACC CTG TCT TTG TTT CCA GGG GAA
48
Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Phe Pro Gly Glu
  1   5   10   15

AGA GCC ACT CTC TCC TGC AGG GCC AGT CAG AGA ATT AGC ACC AGT TTC
96
Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Ile Ser Thr Ser Phe
             20   25   30

TTA GCC TGG TAC CAG CAG AAG CCT GGC CAG TCT CCC AGG CTC CTC ATC
144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Arg Leu Leu Ile
         35   40   45

TTT GAT GCA TCC ACC AGG GCC CCT GGC ATC CCT GAC AGG TTC AGT GCC
192
Phe Asp Ala Ser Thr Arg Ala Pro Gly Ile Pro Asp Arg Phe Ser Ala
     50   55   60

AGT TGG TCT GGG ACA GAC TTC ACT CTC ACC ATC AGC AGA CTG GAG CCT
240
Ser Trp Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro
 65   70   75   80

GAA GAT TTT GCA GTC TAT TAC TGT CAA CAT TAT GGT GGG TCT CCC TGG
288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Tyr Gly Gly Ser Pro Trp
                 85   90   95

ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAG CGA ACT GTG GCT GCA
336
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
                100   105   110

CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA

                              69

384
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115   120   125

ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC
432
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
        130   135   140

AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG
480
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145   150   155   160

GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC
528
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165   170   175

AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC
576
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
                180   185   190

GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC
624
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
            195   200   205

TTC AAC AGG GGA GAG TGT   642
Phe Asn Arg Gly Glu Cys
        210

(2) INFORMATION FOR SEQ ID NO:6:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 214 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Phe Pro Gly Glu
 1   5   10   15

Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Ile Ser Thr Ser Phe
            20   25   30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Arg Leu Leu Ile
        35   40   45

Phe Asp Ala Ser Thr Arg Ala Pro Gly Ile Pro Asp Arg Phe Ser Ala

70

                    50   55   60

Ser Trp Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro
    65   70   75   80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Tyr Gly Gly Ser Pro Trp
                        85   90   95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
                100   105   110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
            115   120   125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
        130   135   140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145   150   155   160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165   170   175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180   185   190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195   200   205

Phe Asn Arg Gly Glu Cys
    210

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 645 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: circular

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapiens
        (F) TISSUE TYPE: Gut-associated lymphoid
        (G) CELL TYPE: Lymphocyte

    (vii) IMMEDIATE SOURCE:
        (B) CLONE: 5-4

    (ix) FEATURE:

           (A) NAME/KEY: CDS
           (B) LOCATION: 1..645
           (D) OTHER INFORMATION: /codon_start= 1
                    /product= "Kappa Light Chain of ANCA associated
                    with Ulcerative Colitis"

     (ix) FEATURE:
           (A) NAME/KEY: misc_RNA
           (B) LOCATION: 1..3
           (D) OTHER INFORMATION: /label= N-TerminalTag

     (ix) FEATURE:
           (A) NAME/KEY: misc_RNA
           (B) LOCATION: 4..285
           (D) OTHER INFORMATION: /label= VKSEGMENT
                    /note= ""VKSEGMENT" refers to Variable Segment of
                    the Kappa Light Chain"

     (ix) FEATURE:
           (A) NAME/KEY: misc_RNA
           (B) LOCATION: 286..327
           (D) OTHER INFORMATION: /label= JK
                    /note= ""JK" refers to Joining Segment of the
                    Kappa Light Chain"

     (ix) FEATURE:
           (A) NAME/KEY: misc_RNA
           (B) LOCATION: 328..645
           (D) OTHER INFORMATION: /label= CK
                    /note= ""CK" refers to Constant Segment of the
                    Kappa Light Chain"

     (ix) FEATURE:
           (A) NAME/KEY: misc_RNA
           (B) LOCATION: 4..66
           (D) OTHER INFORMATION: /label= FR1
                    /note= ""FR1" refers to Framework Region 1"

     (ix) FEATURE:
           (A) NAME/KEY: misc_RNA
           (B) LOCATION: 67..102
           (D) OTHER INFORMATION: /label= CDR1
                    /note= ""CDR1" refers to Complimentarity
                    Determining Region 1"

     (ix) FEATURE:
           (A) NAME/KEY: misc_RNA
           (B) LOCATION: 103..147
           (D) OTHER INFORMATION: /label= FR2
                    /note= ""FR2" refers to Framework Region 2"

     (ix) FEATURE:
           (A) NAME/KEY: misc_RNA
           (B) LOCATION: 148..168
           (D) OTHER INFORMATION: /label= CDR2

                        /note= ""CDR2" refers to Complimentarity
                        Determining Region 2"

        (ix) FEATURE:
              (A) NAME/KEY: misc_RNA
              (B) LOCATION: 169..264
              (D) OTHER INFORMATION: /label= FR3
                    /note= ""FR3" refers to Framework Region 3"

        (ix) FEATURE:
              (A) NAME/KEY: misc_RNA
              (B) LOCATION: 265..294
              (D) OTHER INFORMATION: /label= CDR3
                    /note= ""CDR3" refers to Complimentarity
                    Determining Region 3"

        (ix) FEATURE:
              (A) NAME/KEY: misc_RNA
              (B) LOCATION: 295..327
              (D) OTHER INFORMATION: /label= FR4
                    /note= ""FR4" refers to Framework Region 4"

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCC GAG CTC ACG CAG TCT CCA GGC ACC CTG TCT TTG TCT CCA GGG GAA
48
Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu
  1   5   10   15

AGA GCC ACC CTC TCC TGC AGG GCC AGT CAG GGT GTT AGC AGC GGC TCC
96
Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Gly Val Ser Ser Gly Ser
             20   25   30

TTA GCC TGG TAT CAG CAG AAA GCT GGC CAG GCT CCC AGG CTC CTC ATC
144
Leu Ala Trp Tyr Gln Gln Lys Ala Gly Gln Ala Pro Arg Leu Leu Ile
         35   40   45

TAT GGT GCA TCC AGG AGG GCC ACT GGC ATC CCA GAC AGG TTC ACT GGC
192
Tyr Gly Ala Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Thr Gly
     50   55   60

AGT GGG TCT GGG ACA GAC TTC ACT CTC ACC ATC ACC AGA CTG GAG CCT
240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Leu Glu Pro
 65   70   75   80

GAA GAT TTT GCA GTG TAT TAC TGT CAG CAG TAT GGT AGC TCC CAG GGA
288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Gln Gly
             85   90   95

```
TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT CTC AAA CGA ACT GTG GCT
336
Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Leu Lys Arg Thr Val Ala
         100                 105                 110

GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT
384
Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
         115                 120                 125

GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG
432
Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
     130                 135                 140

GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC
480
Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC
528
Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                 165                 170                 175

AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC
576
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
             180                 185                 190

TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG
624
Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
         195                 200                 205

AGC TTC AAC AGG GGA GAG TGT                                      645
Ser Phe Asn Arg Gly Glu Cys
         210                 215
```

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 215 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu
  1                 5                 10                 15

Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Gly Val Ser Ser Gly Ser
```

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                    20                    25                    30
    Leu Ala Trp Tyr Gln Gln Lys Ala Gly Gln Ala Pro Arg Leu Leu Ile
            35                    40                    45

    Tyr Gly Ala Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Thr Gly
            50                    55                    60

    Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Leu Glu Pro
        65                    70                    75                    80

    Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Gln Gly
                    85                    90                    95

    Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Leu Lys Arg Thr Val Ala
                    100                   105                   110

    Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
            115                   120                   125

    Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
        130                   135                   140

    Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
    145                   150                   155                   160

    Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                    165                   170                   175

    Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
                    180                   185                   190

    Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
            195                   200                   205

    Ser Phe Asn Arg Gly Glu Cys
        210                   215
```

(2) INFORMATION FOR SEQ ID NO:9:

      (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 30 base pairs
           (B) TYPE: nucleic acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

     (iv) ANTI-SENSE: NO

     (ix) FEATURE:
           (A) NAME/KEY: misc_RNA

                    (B) LOCATION: 1..30
                    (D) OTHER INFORMATION: /label= CG1z
                        /note= ""CG1z" refers to the cDNA Primer for IgG1
                        Heavy Chain Constant Segments"

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCATGTACTA GTTTTGTCAC AAGATTTGGG   30

(2) INFORMATION FOR SEQ ID NO:10:

          (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 24 base pairs
                    (B) TYPE: nucleic acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: cDNA

          (iii) HYPOTHETICAL: NO

          (iv) ANTI-SENSE: NO

          (ix) FEATURE:
                    (A) NAME/KEY: misc_RNA
                    (B) LOCATION: 1..24
                    (D) OTHER INFORMATION: /label= VH1a
                        /note= ""VH1a" refers to the cDNA Primer for
                        Variable Segments of the Heavy Chain that are
                        Members of the VH1 Gene Family"

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CAGGTGCAGC TCGAGCAGTC TGGG   24

(2) INFORMATION FOR SEQ ID NO:11:

          (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 24 base pairs
                    (B) TYPE: nucleic acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: cDNA

          (iii) HYPOTHETICAL: NO

          (iv) ANTI-SENSE: NO

          (ix) FEATURE:
                    (A) NAME/KEY: misc_RNA

            (B) LOCATION: 1..24
            (D) OTHER INFORMATION: /label= VH3a
                  /note= ""VH3a" refers to the cDNA Primer for
                  Variable Segments of the Heavy Chain that are
                  Members of the VH3 Gene Family"

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAGGTGCAGC TCGAGGAGTC TGGG   24

(2) INFORMATION FOR SEQ ID NO:12:

      (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 23 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA

      (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO

      (ix) FEATURE:
            (A) NAME/KEY: misc_RNA
            (B) LOCATION: 1..23
            (D) OTHER INFORMATION: /label= VH2f
                  /note= ""VH2f" refers to the cDNA Primer for
                  Variable Segments of the Heavy Chain that are
                  Members of the VH2 Gene Family"

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CAGGTGCAGC TACTCGAGTC GGG   23

(2) INFORMATION FOR SEQ ID NO:13:

      (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 24 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA

      (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO

      (ix) FEATURE:

                    (A) NAME/KEY: misc_RNA
                    (B) LOCATION: 1..24
                    (D) OTHER INFORMATION: /label= VH3f
                            /note= ""VH3f" refers to the cDNA Primer for
                            Variable Segments of the Heavy Chain that are
                            Members of the VH3 Gene Family"

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GAGGTGCAGC TGCTCGAGTC TGGG   24

(2) INFORMATION FOR SEQ ID NO:14:

        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 23 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: cDNA

        (iii) HYPOTHETICAL: NO

        (iv) ANTI-SENSE: NO

        (ix) FEATURE:
                (A) NAME/KEY: misc_RNA
                (B) LOCATION: 1..23
                (D) OTHER INFORMATION: /label= VH4f
                        /note= ""VH4f" refers to the cDNA Primer for
                        Variable Segments of the Heavy Chain that are
                        Members of the VH4 Gene Family"

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CAGGTGCAGC TGCTCGAGTC GGG   23

(2) INFORMATION FOR SEQ ID NO:15:

        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 23 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: cDNA

        (iii) HYPOTHETICAL: NO

        (iv) ANTI-SENSE: NO

(ix) FEATURE:
    (A) NAME/KEY: misc_RNA
    (B) LOCATION: 1..23
    (D) OTHER INFORMATION: /label= VH6a
        /note= ""VH6a" refers to the cDNA Primer for
        Variable Segments of the Heavy Chain that are
        Members of the VH6 Gene Family"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAGGTACAGC TCGAGCAGTC AGG   23

(2) INFORMATION FOR SEQ ID NO:16:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 27 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 1..27
        (D) OTHER INFORMATION: /label= VH6f
            /note= ""VH6f" refers to the cDNA Primer for
            Variable Segments of the Heavy Chain that are
            Members of the VH6 Gene Family"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CAGGTACAGC TGCTCGAGTC AGGTCCA   27

(2) INFORMATION FOR SEQ ID NO:17:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 58 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

(ix) FEATURE:
     (A) NAME/KEY: misc_RNA
     (B) LOCATION: 1..58
     (D) OTHER INFORMATION: /label= CK1d
         /note= ""CK1d" refers to the cDNA Primer for Kappa
         Light Chain Constant Segments"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCGCCGTCTA GAATTAACAC TCTCCCCTGT TGAAGCTCTT TGTGACGGGC
GAACTCAG   58

(2) INFORMATION FOR SEQ ID NO:18:

    (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 24 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (ix) FEATURE:
     (A) NAME/KEY: misc_RNA
     (B) LOCATION: 1..24
     (D) OTHER INFORMATION: /label= VK1a
         /note= ""VK1a" refers to the cDNA Primer for
         Variable Segments of the Kappa Light Chain that
         are Members of the VK1 Gene Family"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GACATCGAGC TCACCCAGTC TCCA   24

(2) INFORMATION FOR SEQ ID NO:19:

    (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 24 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

```
    (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 1..24
        (D) OTHER INFORMATION: /label= VK2a
            /note= ""VK2a" refers to the cDNA Primer for
            Variable Segments of the Kappa Light Chain that
            are Members of the VK2 Gene Family"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GATATTGAGC TCACTCAGTC TCCA                                               24

(2) INFORMATION FOR SEQ ID NO:20:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 24 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO


    (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 1..24
        (D) OTHER INFORMATION: /label= VK3a
            /note= ""VK3a" refers to the cDNA Primer for
            Variable Segments of the Kappa Light Chain that
            are Members of the VK3 Gene Family"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GAAATTGAGC TCACGCAGTC TCCA                                               24

(2) INFORMATION FOR SEQ ID NO:21:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 22 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA


    (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 1..22
```

(D) OTHER INFORMATION: /note= "5' Heavy Chain Sequencing
        Primer"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGCCGCAAAT TCTATTTCAA GG   22

(2) INFORMATION FOR SEQ ID NO:22:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 18 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (ix) FEATURE:
            (A) NAME/KEY: misc_RNA
            (B) LOCATION: 1..18
            (D) OTHER INFORMATION: /note= "3' Heavy Chain Sequencing
                Primer"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGCTGTGCCC CCAGAGGT   18

(2) INFORMATION FOR SEQ ID NO:23:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 18 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (ix) FEATURE:
            (A) NAME/KEY: misc_RNA
            (B) LOCATION: 1..18
            (D) OTHER INFORMATION: /note= "5' Light Chain Sequencing
                Primer"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTAAACTAGC TAGTCGCC   18

(2) INFORMATION FOR SEQ ID NO:24:

        (i) SEQUENCE CHARACTERISTICS:

```
        (A) LENGTH: 21 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA


   (ix) FEATURE:
        (A) NAME/KEY: misc_RNA
        (B) LOCATION: 1..21
        (D) OTHER INFORMATION: /note= "3' Light Chain Sequencing
            Primer"


   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATAGAAGTTG TTCAGCAGGC A                                            21
```

We claim:

1.   A method of measuring the presence or absence of
perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA)
associated with ulcerative colitis, primary sclerosing
5   cholangitis, or type 1 autoimmune hepatitis in a sample,
said method comprising:
       (a)   contacting the sample and a detectable
secondary antibody with fixed neutrophils under
conditions suitable to form a complex of neutrophil, p-
10   ANCA and detectable secondary antibody,
             wherein said fixed neutrophils are subjected
       to DNase under conditions sufficient to cause
       substantially complete digestion of cellular DNA
       without significant loss of nuclear or cellular
15   morphology prior to said contacting step, and
       wherein said secondary antibody has specificity
       for the class determining portion of p-ANCA;
       (b)   separating unbound secondary antibody from
the complex;
20   (c)   assaying for the pattern of p-ANCA
immunoreactivity by detecting the presence, absence or
pattern of complexed secondary antibody, compared to a
control,
             wherein said control is the result of
25   repeating the present method on a sample from the
       same source, except that the neutrophils are not
       subjected to DNase.

2.   The method of claim 1, wherein said fixed
neutrophils are alcohol-fixed neutrophils.

30   3.   The method of claim 2, wherein said alcohol-fixed
neutrophils are methanol-fixed neutrophils.

4.   A method according to claim 3, wherein the
conditions sufficient to cause substantially complete
digestion of cellular DNA, without substantial loss of
nuclear or cellular morphology, comprise incubating said
5   neutrophils in a concentration of DNase of about 2 to 10
units of DNase per milliliter of buffer for a time in the
range of about 15 minutes to one hour at a temperature in
the range of about 22°C to 40°C.

5.   A method according to claim 3, wherein methanol-
10 fixed neutrophils are contacted with 3 units of DNase per
milliliter buffer for 30 minutes at 37°C.

6.   A method according to claim 1, wherein the
secondary antibody is anti-IgG.

7.   A method according to claim 1, wherein the
15 neutrophils are human neutrophils, the sample is human blood
serum and the secondary antibody is anti-human IgG.

8.   A method according to claim 1, wherein the
secondary antibody is a combination of antibodies, wherein
at least one secondary antibody has specificity for the
20 class determining portion of p-ANCA and at least one
secondary antibody is detectable.

9.   A method according to claim 8, wherein the
neutrophils are human neutrophils, the sample is human blood
serum, one of the secondary antibodies is mouse anti-human
25 IgG and one of the secondary antibodies is detectable goat
anti-mouse IgG.

10.   A method according to claim 1, wherein the secondary antibody is detectable by detecting enzymatic conversion, radioactivity, fluorescence or color.

11.   A method according to claim 10, wherein the secondary antibody is chemically linked to an enzyme.

12.   A method according to claim 11, wherein the enzyme is alkaline phosphatase and the substrate for enzymatic conversion is para-nitrophenyl phosphate.

13.   A method according to claim 10, wherein the secondary antibody is chemically linked to a fluorogenic substance.

14.   A method according to claim 13, wherein the fluorogenic substance is fluorescein isothiocyanate.

15.   A method of detecting the presence of perinuclear anti-neutrophil cytoplasmic antibody (p-ANCA) associated with type 1 autoimmune hepatitis in a sample, comprising:
           (a)   contacting fixed neutrophils with a sample and a detectable secondary antibody under conditions suitable to form an immune complex of neutrophil, p-ANCA and detectable secondary antibody,
                wherein cellular DNA of the fixed neutrophils has been digested by DNase without significant loss of nuclear or cellular morphology, and
                wherein the detectable secondary antibody is detectable by fluorescence and is specific for the class determining portion of p-ANCA;
           (b)   separating unbound secondary antibody from the immune complex; and

(c) detecting the immunofluorescent staining
pattern of the complex as compared to a control,
wherein the control is the result of
repeating the present method using fixed
5   neutrophils wherein the cellular DNA of the fixed
neutrophils has not been digested by DNase, and
wherein the presence of a granular
cytoplasmic staining pattern in the sample, and a
perinuclear staining pattern in the control,
10   indicates the presence of p-ANCA associated with
type 1 autoimmune hepatitis in the sample.

16.   The method of claim 15, wherein said fixed
neutrophils are alcohol-fixed neutrophils.

17.   The method of claim 16, wherein said alcohol-fixed
15 neutrophils are methanol-fixed neutrophils.

18.   A method according to claim 17, wherein the
conditions sufficient to cause substantially complete
digestion of cellular DNA, without substantial loss of
nuclear or cellular morphology, comprise incubating said
20 neutrophils in a concentration of DNase of about 2 to 10
units of DNase per milliliter of buffer for a time in the
range of about 15 minutes to one hour at a temperature in
the range of about 22°C to 40°C.

19.   A method according to claim 18, wherein the
25 secondary antibody is anti-IgG.

20.   A method of detecting the presence of perinuclear
anti-neutrophil cytoplasmic antibody (p-ANCA) associated
with primary sclerosing cholangitis in a sample, comprising:

(a) contacting fixed neutrophils with a sample
and a detectable secondary antibody under conditions
suitable to form an immune complex of neutrophil, p-
ANCA and detectable secondary antibody,

5   wherein cellular DNA of the fixed neutrophils
has been digested by DNase without significant
loss of nuclear or cellular morphology, and
            wherein the detectable secondary antibody is
detectable by fluorescence and is specific for the

10   class determining portion of p-ANCA;
       (b) separating unbound secondary antibody from
the immune complex; and
       (c) detecting the immunofluorescent staining
pattern of the complex as compared to a control,

15   wherein the control is the result of
repeating the present method using fixed
neutrophils wherein the cellular DNA of the fixed
neutrophils has not been digested by DNase, and
            wherein the presence of a homogenous

20   cytoplasmic staining pattern in the sample, and a
perinuclear staining pattern in the control,
indicates the presence of p-ANCA associated with
primary sclerosing cholangitis in the sample.

       21. The method of claim 20, wherein said fixed
25   neutrophils are alcohol-fixed neutrophils.

       22. The method of claim 21, wherein said alcohol-fixed
neutrophils are methanol-fixed neutrophils.

       23. A method according to claim 22, wherein the
conditions sufficient to cause substantially complete
30   digestion of cellular DNA, without substantial loss of
nuclear or cellular morphology, comprise incubating said

neutrophils in a concentration of DNase of about 2 to 10
units of DNase per milliliter of buffer for a time in the
range of about 15 minutes to one hour at a temperature in
the range of about 22°C to 40°C.

5   24.   A method according to claim 23, wherein the
secondary antibody is anti-IgG.

           25.   A method of differentiating primary sclerosing
cholangitis from type 1 autoimmune hepatitis, comprising:
                  (a)   contacting fixed neutrophils with a sample
10 and a detectable secondary antibody under conditions
suitable to form an immune complex of neutrophil, p-
ANCA and detectable secondary antibody,
                         wherein cellular DNA of the fixed neutrophils
has been digested by DNase without significant
15   loss of nuclear or cellular morphology, and
                         wherein the detectable secondary antibody is
detectable by fluorescence and is specific for the
class determining portion of p-ANCA;
                  (b)   separating unbound secondary antibody from
20 the immune complex; and
                  (c)   detecting the immunofluorescent staining
pattern of the complex as compared to a control,
                         wherein the control is the result of
repeating the present method using fixed
25   neutrophils wherein the cellular DNA of the fixed
neutrophils has not been digested by DNase, and
                         wherein the presence of a homogeneous
cytoplasmic staining pattern in the sample and a
perinuclear staining pattern in the control
30   indicates primary sclerosing cholangitis, and
                         wherein the presence of a granular
cytoplasmic staining pattern in the sample and a

perinuclear staining pattern in the control
indicates type 1 autoimmune hepatitis.

26. The method of claim 25, wherein said fixed
neutrophils are alcohol-fixed neutrophils.

27. The method of claim 26, wherein said alcohol-fixed
neutrophils are methanol-fixed neutrophils.

28. A method according to claim 27, wherein the
conditions sufficient to cause substantially complete
digestion of cellular DNA, without substantial loss of
nuclear or cellular morphology, comprise incubating said
neutrophils in a concentration of DNase of about 2 to 10
units of DNase per milliliter of buffer for a time in the
range of about 15 minutes to one hour at a temperature in
the range of about 22°C to 40°C.

29. A method according to claim 28, wherein the
secondary antibody is anti-IgG.

30. A method of differentiating ulcerative colitis,
primary sclerosing cholangitis, Crohn's disease and type 1
autoimmune hepatitis, said method comprising:
        (a) contacting fixed neutrophils with a sample
    and a detectable secondary antibody under conditions
    suitable to form an immune complex of neutrophil, p-
    ANCA and detectable secondary antibody,
            wherein cellular DNA of the fixed neutrophils
        has been digested by DNase without significant
        loss of nuclear or cellular morphology, and
            wherein the detectable secondary antibody is
        detectable by fluorescence and is specific for the
        class determining portion of p-ANCA;

(b)   separating unbound secondary antibody from the immune complex; and

(c)   detecting the immunofluorescent staining pattern of the complex as compared to a control,

wherein the control is the result of repeating the present method using fixed neutrophils wherein the cellular DNA of the fixed neutrophils has not been digested by DNase, and

wherein loss of detectable complex associated with perinuclear staining pattern, as compared to said control, indicates ulcerative colitis,

wherein conversion of detectable complex associated with perinuclear staining pattern to homogenous cytoplasmic staining pattern, as compared to said control, indicates primary sclerosing cholangitis,

wherein conversion of detectable complex associated with perinuclear staining pattern to granular cytoplasmic staining pattern, as compared to said control, indicates type 1 autoimmune hepatitis, and

wherein absence of a detectable complex associated with perinuclear staining pattern in said control indicates Crohn's disease.

31.   A kit for detecting the presence or absence of perinuclear anti-neutrophil cytoplasmic antibody of ulcerative colitis, primary sclerosing cholangitis or type 1 autoimmune hepatitis in a sample, comprising neutrophils and DNase.

32. The kit according to claim 31, further comprising a detectable secondary antibody having specificity for the class determining portion of p-ANCA.

33. The kit of claim 31, wherein said neutrophils are alcohol fixed neutrophils.

34. The kit of claim 33, wherein said neutrophils are immobilized.

35. The kit of claim 34, wherein the neutrophils are immobilized on a solid substrate selected from the group consisting of microtiter plates, beads, and glass slides.

36. The kit according to claim 35, further comprising a detectable secondary antibody having specificity for the class determining portion of p-ANCA.

37. A kit for measuring the presence or absence of perinuclear anti-neutrophil cytoplasmic antibody of ulcerative colitis, primary schlerosing colangitis or type I autoimmune hepatitis in a sample, comprising DNase-treated neutrophils and a detectable secondary antibody having specificity for the class determining portion of p-ANCA.

38. The kit of claim 37, wherein said secondary antibody is anti-IgG.

39. The kit of claim 37, wherein said neutrophils are alcohol fixed.

40. The kit of claim 39, wherein said neutrophils are immobilized.

41. The kit of claim 40, wherein said neutrophils are immobilized on a solid substrate selected from the group consisting of microtiter plates, beads, and glass slides.

42. A method of measuring the presence or absence of perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) associated with ulcerative colitis in a sample, said method comprising:

(a) contacting the sample and a detectable secondary antibody with fixed neutrophils under conditions suitable to form a complex of neutrophil, p-ANCA and detectable secondary antibody,

wherein said fixed neutrophils are subjected to DNase under conditions sufficient to cause substantially complete digestion of cellular DNA without significant loss of nuclear or cellular morphology prior to said contacting step, and wherein said secondary antibody has specificity for p-ANCA associated with ulcerative colitis;

(b) separating unbound secondary antibody from the complex;

(c) assaying for the pattern of p-ANCA immunoreactivity by detecting the presence, absence or pattern of complexed secondary antibody, compared to a control,

wherein said control is the result of repeating the present method on a sample from the same source, except that the neutrophils are not subjected to DNase.
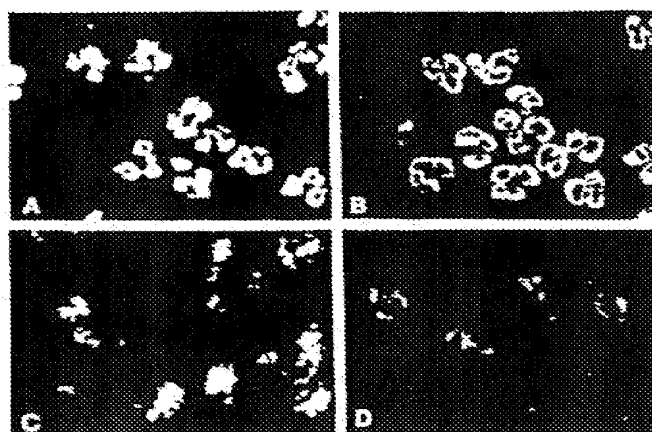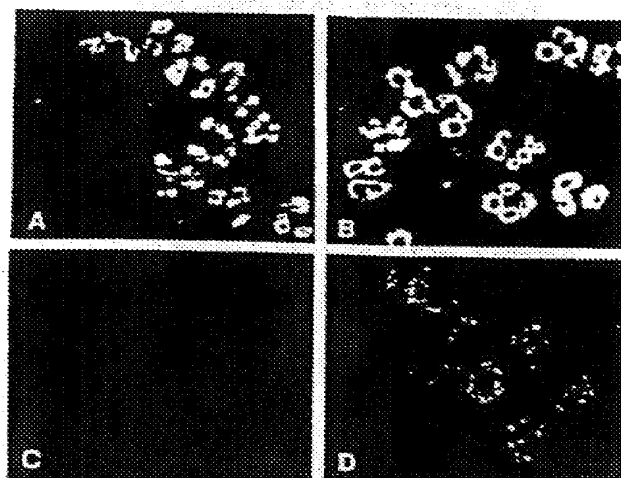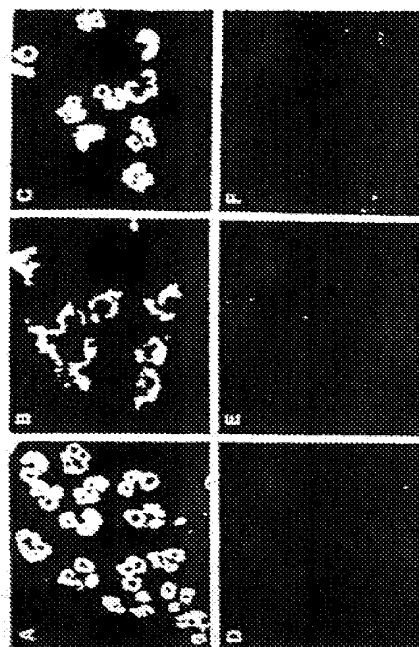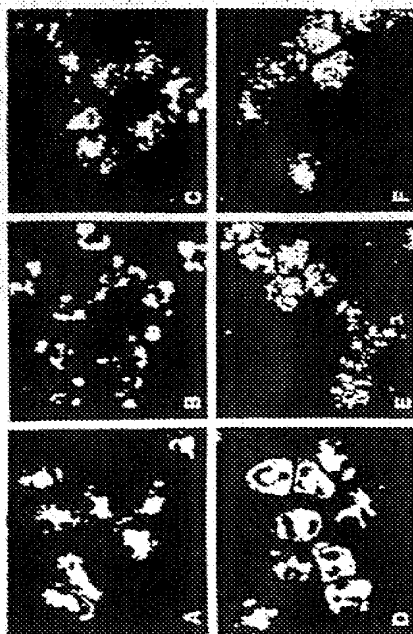
93

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :Please See Extra Sheet.

US CL   :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	EP, 0,615,129 A1 (CEDARS-SINAI MEDICAL CENTER) 14 September 1994, see entire document.	1-14, 20-24, 31-42 --------- 15-19, 25-30
X,P	EGGENA et al. Phage Display Cloning and Characterization of an Immunogenic Marker (Perinuclear Anti-Neutrophil Cytoplasmic Antibody) in Ulcerative Colitis. American Association of Immunologists. 15 May 1996, Volume 156, No. 10, pages 4005-4011, especially pages 4007-4008.	1-42

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 JULY 1996	04 SEP 1996

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	CAROL A. SPIEGEL
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	VIDRICH et al. Segregation of pANCA Antigenic Recognition by DNase Treatment of Neutrophils: Ulcerative Colitis, Type I Autoimmune Hepatitis, and Primary Sclerosing Cholangitis. Journal of Clinical Immunology. November 1995, Volume 15, No. 6, pages 293-299, especially pages 294-297.	1-42
Y	TARGAN et al. High-Titer Antineutrophil Cytoplasmic Antibodies in Type-1 Autoimmune Hepatitis. Gastroenterology. April 1995, Volume 108, No. 4, pages 1159-1166, see especially pages 1161-1163.	1-42
Y	MONESTIER et al. Deoxyribonuclease I treatment of histones for the detection of anti-histone antibodies in solid-phase immunoassays. Journal of Immunological Methods. 1990, Volume 127, No. 2, pages 289-291, see entire article.	1-42
Y, P	EGGENA et al. Histone H1: The Ulcerative Colitis Specific pANCA Target Antigen. FASEB Journal. 30 April 1996, Volume 10, No. 6, page A1079, see abstract 463.	1-42
Y, P	BILLING et al. Nuclear Localization of the Antigen Detected by Ulcerative Colitis-Associated Perinuclear Antineutrophil Cytoplasmic Antibodies. American Journal of Pathology. October 1995, Volume 147, No. 4, pages 979-987, see especially pages 983-986.	1-42
Y	GUR et al. Autoantibody Profile of Primary Sclerosing Cholangitis. Pathobiology. March-April 1995, Volume 63, pages 76-82, see entire article.	1-15, 20-42
Y	RUMP et al. A New Type of Perinuclear Anti-Neutrophil Cytoplasmic Antibody (p-ANCA) in Active Ulcerative Colitis but not in Crohn's Disease. Immunobiology. 1990, Volume 181, No. 4/5, pages 406-413, see especially pages 408, 411-412.	1-14, 30-42
Y	SAXON et al. A distinct subset of antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. Journal of Allergy and Clinical Immunology. August 1990, Volume 86, No. 2, pages 202-210, see especially pages 203-204.	1-42

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

G01N 33/563, 33/569, 33/567, 33/564, 33/53

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

436/506, 508, 519, 63; 435/7.1, 7.24, 7.9, 7.92, 40.51

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

436/506, 508, 519, 63, 518, 808, 811, 825; 435/7.1, 7.24, 7.9, 7.92, 40.51, 6, 961, 962, 975

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

DIALOG, APS, JPO Databases
search terms: histone H1, autoimmune, antibody, colitis, hepatitis, cholangitis, DNase, pANCA, ANCA, anti-neutrophil, cytoplasmic